DISCLOSURE OF GENETIC VARIANTS OF UNCERTAIN SIGNIFICANCE RESULTS IN AN EXOME COHORT

by

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ABSTRACT

Background: The emergence of next generation sequencing as a clinical tool in aiding diagnosis is fueling enthusiasm to provide genomic information to patients. One consequence of this technology is that genetic results are more likely to include variants of uncertain significance (VUS). VUS are alterations in the DNA sequence of a gene that have an intermediate probability of pathogenicity or disease risk. There are no universally accepted standards for reporting VUS results. Also, conveying the potential implications of uncertain results to patients and research participants sufficiently to inform their decision-making is challenging. Uncertainty can have a variety of psychological effects. Perceptions related to uncertainty are likely to predict decisions to learn genetic results and to act on the information. There is a need to examine the impact of the inherent uncertainty in genetic sequencing information on the perceptions of recipients. However, most reports in the literature involve hypothetical studies of VUS disclosures.

Objectives: a) To describe the effect of perceived risk and perceived severity associated with disclosure of genetic variants of uncertain significance results on health behavior intentions, b) to describe the effect of perceived value of information and self-efficacy associated with disclosure of genetic variants of uncertain significance results on health behavior intentions, c) to examine the influence of resilience, optimism, and tolerance of uncertainty on health behavior intentions post disclosure of genetic variants of uncertain significance results, and d) to measure the level of regret associated with decision to learn genetic variants of uncertain significance results 2 weeks post disclosure.

Design and Methods: A predictive correlational design was employed. Survey responses were collected 2 weeks following disclosure of results. Correlation analysis and multiple linear regressions were used to examine the relationships of interest in the first three aims, while descriptive statistics was used for the fourth aim.

Sample: Eighty-one participants with exome-generated variants of uncertain significance (VUS) in one of 20 cardiomyopathy-associated genes from the ClinSeq® study participated in the study.

Findings: Most participants (79%) intended to seek more information about their result in the future, over 80% intended to share their result with family members and health-care providers, and 46% intended to use result to change their lifestyle and health behaviors. Perceived risk, perceived severity, perceived value of information, self-efficacy, and sex explained 42.5% (Adjusted R^2 = 38.6%) of the variance in health behavior intentions of recipients of VUS results. Perceived value of information was the strongest predictor of health behavior intentions (β = 0.529, $p < 0.001$). Behavior intentions was moderately correlated with optimism ($r = 0.25$, $p = 0.03$), and weakly correlated with resilience ($r = 0.11$, $p = 0.614$) and tolerance of uncertainty ($r = -0.05$, $p = 0.669$). The mean decision regret score was 12.41 (SD=16.42) on a 0 to 100 scale (score of 0 = no regret). Thirty-five participants (51.5%) reported no regret (0/100) associated with their decision, and 57 (83.8%) reported a score of 30/100 or less.

Conclusions: Our findings suggest that perceived benefits associated with receipt of uncertain genetic information are more likely to motivate recipients to pursue health related behaviors than discussions of possible risk and severity associated with results. Despite the ambiguity

associated with genetic variants of uncertain significance, there is minimal regret regarding a decision to learn these results.

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DEDICATIONS

To my wife, Tara Brooke Lawal,

for her constant support and prayers

and

My children

Mariah, Micah, Braylon, and Brielle

For their inspiration and sacrifice, in obvious and not so obvious ways

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Chapter One: Introduction

Background

Developments in next-generation sequencing technologies are expected to enable a more personalized approach to patient care and disease risk stratification. The use of next generation sequencing technology is increasingly becoming accessible to clinical and research laboratories. Next generation sequencing can identify the genetic cause of a disease, but can also identify variants underlying diseases that are not being sought (secondary or incidental findings). This increase in genomic interrogation has led to ongoing debates focusing on whether incidental findings should be returned to research participants (Wolf et al., 2012; Ferriere & Van Ness, 2012). Although a few are expected to be clinically significant, the risk consequences (whether the variant is associated with a phenotype) of most identified secondary variants are often unknown and therefore designated as variants of uncertain significance (VUS). Analysis of the Myriad Genetics Laboratory clinical testing data on *BRCA1* and *BRCA2* revealed that sequencing-based genetic testing has a similar likelihood of returning a VUS result (13%) as a deleterious mutation (15.1%) (Frank et al. 2002), with other testing laboratories reporting estimates up to 15% - 21% of alterations as VUSs in these well studied genes (Lindor et al., 2013; Ready et al., 2011). The likelihood of a VUS result is even higher for individuals from understudied populations who undergo genetic testing, partly due to limited information on the rare variants in that population (John et al., 2007; Nanda et al., 2005). The growing efforts to personalize healthcare is driving the need to help researchers communicate the uncertainties of genetic results to participants amidst the limited understanding and fragmented body of knowledge available on VUS interpretations (Han et al., 2011). Communication of uncertainty regarding disease

risk estimates has complex effects including distress consistent with ambiguity aversion and distortion of recipients' risk perception. According to Han and colleagues, these responses are influenced by the recipients' personality type and perceived credibility and value of disclosed information (Han et al., 2011).

Due to the uncertainty surrounding the implications of VUS's and the challenge of accurate risk communication to probands and their relatives for intervention or surveillance, there are currently no commonly accepted guidelines for providing clinical interpretation and recommendations for those receiving VUS results (Vink, van Asperen, Devilee, Breuning, & Bakker, 2005). Although areas of variability have been documented on whether or not VUS results should be disclosed to research participants, there is little controversy about the public's interest to learn about their individual sequencing information (Graves et al., 2014). This is evidenced by the rapidly growing interest in direct-to-consumer genotyping companies in spite of widely variable interpretations of the disclosed data, and reports that genetic findings can benefit participants through positive health outcomes (McGuire & Lupinski, 2010). However, the benefits associated with ambiguous medical test results such as VUS are poorly defined due to the uncertainty associated with their pathogenicity. Additionally, there is a lack of information describing how recipients interpret VUS results, which makes it challenging to infer what sort of personal meaning individuals might attribute to this test result.

Uncertainty surrounding health risks can affect disease perceptions and change how one interprets risk with regards to developing disease. Personality traits such as resilience, optimism, and tolerance of uncertainty have been documented to influence perceptions of risk, disease severity, benefits of taking action, and self-efficacy. To build resilience, an individual must be exposed to adversity or a threat to physical or psychological health. Despite this adversity or threat, the individual

adapts to minimize or avoid a risk (Luthar, Cicchetti, & Becker, 2000). Resilience is resistance, recovery, or rebound of mental and physical health after a challenge, which results from an individual's interaction with societal, community, family, physiological, and cellular factors across the life course (Szanton & Gill, 2010). Resilience may be influenced by genetics, exposure and experience with adversity, the desire to succeed, and social support (Luthar, Cicchetti, & Becker, 2000; Szanton & Gill, 2010; Dyer & McGuiness, 1996). The tendency to hold optimistic beliefs about the future has been associated with better engagement in health-related behaviors in several prospective studies, leading to lower incidence of cardiovascular disease (Kubzansky, Sparrow, Vokonas, & Kawachi, 2001), better prognosis following heart surgery (Scheier et al., 1999), and greater longevity (Giltay, Kamphuis, Kalmijin, Zitman, & Kromhout, 2006). Worry can be defined as concern about future events in which there is uncertainty about the outcome. Current research suggests that tolerance of uncertainty may be important in understanding worry and may play a role in the etiology and maintenance of worry. Tolerance of uncertainty is the tendency of an individual to consider it acceptable that a negative event may occur (Dugas, Gosselin, & Ladouceur, 2001). These personality traits indirectly influence health-related behaviors by influencing an individual's perceptions. There is limited information on the impact of VUS disclosure on risk perception, perceived severity, and intentions to pursue health-related behavior. This phenomenon remains unexplored in genes associated with cardiomyopathy and arrhythmia susceptibility.

This study was part of an ongoing genetics study (ClinSeq®) at the National Institutes of Health (NIH). ClinSeq® is a longitudinal study of >1000 individuals with a spectrum of atherosclerosis from unaffected to severe, that have been evaluated by exome or genome sequencing and have a choice about what types of information they want returned to them (Biesecker et al., 2009). The initial focus

of the ClinSeq® study was on atherosclerotic heart disease but a majority are healthy volunteers. ClinSeq® study provides a novel opportunity for baseline assessment of participant preferences to learn about their individual DNA results. Participants are informed of the types of results that can be generated, including limitations in interpreting data, and lack of reporting of uninterpretable information and non-pathogenic variants. Participants completed a baseline survey during the ClinSeq® enrollment about their intentions to receive genetic results, and were asked their general preferences and reasons for receiving results. A prior hypothetical study to learn the relative perceived value of the different categories of genetic findings (gene variants that predispose to treatable disease, untreatable disease, no effect, or VUS) among ClinSeq® participants reported that although there was significantly less interest in VUS, it was not to the degree anticipated. Participants' attitudes and social norms were significantly correlated in all four categories and each was independently correlated with intentions to receive results (Facio et al., 2013). Additionally, information about risks to future generations was viewed by ClinSeq® participants to be as valuable as information about personal health risk that can be mitigated. This hypothetical study concluded that the participants' responses reflect their confidence in the usefulness of sequence information (high response efficacy), even information that is currently not interpretable such as VUS. Response efficacy, defined as confidence in the ability to use information to maximize health, has been found to significantly predict attitudes towards receiving results and attitudes explain a significant amount of the variance in intentions to undergo genetic testing (Wade et al., 2011). An exploratory study of 322 ClinSeq® participants identified enthusiasm for learning all types of results, with expectations to learn more about the genetic factors that contribute to their health risks (Facio et al., 2011). However, the underlying perceived value of learning of VUS

results, perceived risk and severity attributed to uncertain DNA information, and what will be done if such information were received was not adequately investigated.

In an effort to address the issue of returning secondary variants to research participants, Ng and colleagues selected 22 arrhythmia and 41 cardiomyopathy-associated genes, and analyzed exome sequencing data from 870 ClinSeq® participants. Participants were not pre-selected for personal or family history of arrhythmia, cardiomyopathy, or sudden cardiac death (Ng et al., 2013). Nonsense, frameshift, splice-site, and non-synonymous variants were analyzed in 870 participants in 41 genes associated with dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM), two conditions with a frequency of ~1/250 and ~ 1/500 respectively in the general population (Maron et al., 1995). Primary literature identified through the Human Gene Mutation Database (HGMD) and Locus-specific databases (LSDB) were consulted to assign pathogenicity classes according to the criteria in Appendix G.

There is a dearth of literature describing the impact of uncertain genetic results on recipients' health-related behaviors. To address the question of the impact of receiving uncertain genetic findings on research participants, it is important to extend the findings of hypothetical studies to actual participants facing these choices. We returned some of the cardiomyopathy-associated class III (VUS) variants identified by Ng and colleagues to study the impact of returning uncertain DNA results on health-related behaviors.

Using the Health Belief Model (HBM) as a framework, this study examined the impact of receiving cardiomyopathy-associated VUS results on the recipient's: perceived severity and perceived susceptibility (risk perception) to cardiomyopathy; perceived benefits (value) attached to acting on the result by pursuing health-related behaviors, and; perceived self-efficacy (competence) to execute the

desired health-related behaviors. The HBM proposes that perceived susceptibility (risk) to a condition and perceived severity (seriousness) associated with the condition comprises an individual's threat perception, and that threat perception motivates action. According to the HBM, a particular action will only be adopted if the perceived benefits outweigh its perceived barriers. In addition, perceived self-efficacy and cues to action are needed to trigger or stimulate health-related behaviors, while diverse demographic and socio-psychological factors indirectly influence behavior by modifying perceptions (Janz & Becker, 1984). Health-related behavior is an action related to decreasing the risk of a certain disease outcome.

Decision-making, especially in the face of uncertain or equivocal evidence, is sensitive to personal preferences (Elwyn & Miron-Shatz, 2010). Regret is the adverse emotion one experiences with the realization that the current situation would be more favorable if one had chosen differently (Zeelenberg & Pieters, 2007). A key goal of decision-making is to minimize regret. The level of regret following a decision is an effective indicator for assessing the quality of information an individual used to arrive at a decision (Joseph-Williams, Edwards, & Elwyn, 2011; Carere et al., 2016). Regret is a complex emotion that is seldom studied outside the clinical context of oncology (Stacey et al., 2014). Medical related decisions can lead to negative consequences, and insights into perceptions guiding an individual's decision within the context of genetics warrants further investigation.

The purpose of this study was to describe the effects of disclosing cardiomyopathy-associated VUS genetic results on participants' intentions to pursue health-related behaviors. Using the Health Belief Model (HBM), the impact of perceived risk, perceived severity, perceived value of information (benefit), and self-efficacy was used to predict recipients' intentions of pursuing health-related behaviors. The indirect influence of resilience, optimism, and tolerance of uncertainty as modifying

factors on health-related behaviors was also examined. Additionally, the level of regret associated with participants' decision to learn their VUS result was measured. To achieve these goals, the following specific aims were used to guide this study.

Specific Aims

Aim 1: Describe the effect of perceived risk and perceived severity associated with disclosure of genetic VUS results on health behavior intentions.

Hypothesis $_1$: High levels of perceived risk and severity lead to high intentions to pursue health-related behaviors.

Aim 2: Examine the effect of perceived value of information and self-efficacy associated with disclosure of genetic VUS results on health behavior intentions.

Hypothesis $_2$: High levels of perceived information value and self-efficacy increase intentions to pursue health-related behaviors.

Aim 3: Examine the influence of resilience, optimism, and tolerance of uncertainty on health behavior intentions post disclosure of genetic VUS results.

Hypothesis $_3$: Resilience, optimism, and tolerance of uncertainty have a moderating influence on intentions to pursue health-related behaviors.

Aim 4: Measure the level of regret associated with decision to learn genetic VUS results two weeks post-disclosure.

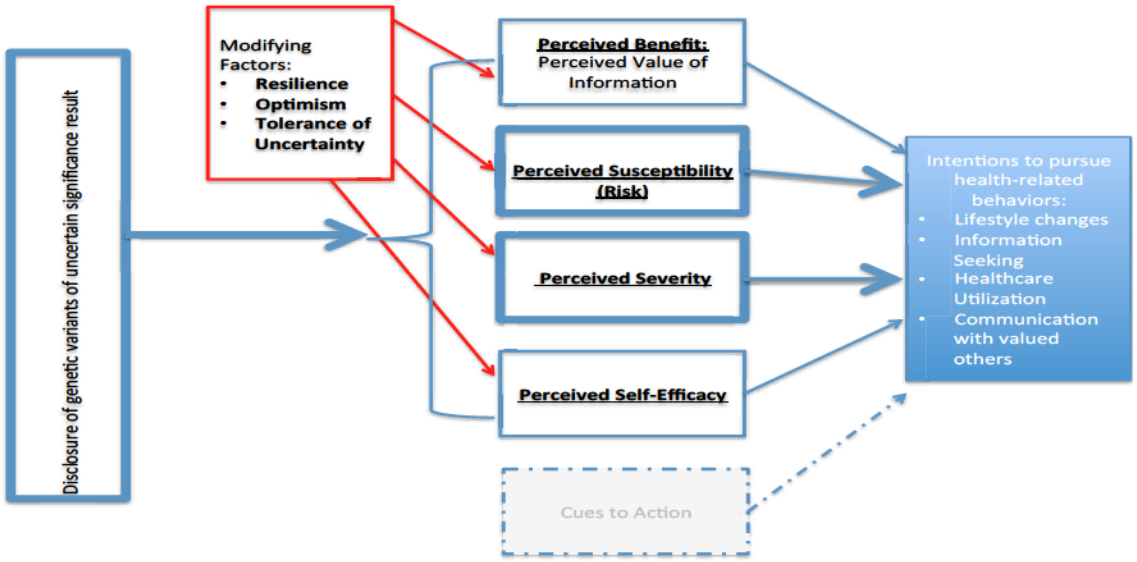
Hypothesis $_4$: The uncertainty associated with genetic VUS information increases regret with decision to learn result.

Theoretical Framework

The Health Belief Model (HBM) was used to guide this study. The HBM specifies that an individual's perceptions of the costs and benefits of an action predict behavior, and that perception of reality rather than objective reality influences behavior. Behavior is regulated by how its consequences are interpreted and understood by the individual. The model argues that a person's belief about their likelihood of getting a disease or condition (susceptibility), their perception of the severity of the negative health outcome, together with belief in the effectiveness of the recommended health behavior (benefits) can predict whether a person will follow a preventive behavior (Rosenstock, 1974). The HBM hypothesizes that health-related actions depend on the occurrence of four factors: the existence of sufficient motivation or concern to make a health issue relevant (seriousness); the belief that one is susceptible to a serious health concern (susceptibility); and the belief that following a particular health recommendation would be beneficial in reducing the perceived threat (benefits); at a subjectively-acceptable cost (barriers that must be overcome to follow the health recommendation) (Rosenstock, Strecher, & Becker, 1988). The combination of susceptibility and severity has been labeled as perceived threat, and a subconscious, cost-benefit analysis occurs when individuals weigh the action's expected benefits with perceived barriers. The model also includes two variables that attempt to capture motivation to pursue healthy behaviors- cues to action and self-efficacy. When a person is motivated and can perceive a beneficial action to take, a cue to action is an internal (e.g. symptoms) or external (media publicity, recommendations by healthcare provider or valued other)

event that prompts an individual to action. Self-efficacy is defined as the conviction that one can successfully execute the behavior required to produce the desired outcome. Self-efficacy is distinctly different from outcome expectation – defined as a person's estimate that a given behavior will lead to a certain outcome – and are both required to drive behavior toward an outcome (Bandura, 1977). For a behavioral change to succeed, people must believe themselves competent to implement that change. There is growing support for the role of self-efficacy in the initiation and maintenance of behavioral change (Bandura, 1977; Janz & Becker, 1984). Although not incorporated into early formulations of the HBM, the addition of self-efficacy recognizes the complexities underlying both behavior and factors that motivate individuals to action. Finally, the HBM assumes that demographic (age, sex, education), psychosocial (personality traits, social class), and structural variables (knowledge about disease, personal/family disease history) can affect perceptions and indirectly influence health-related behaviors (Janz & Becker, 1984). The variables (constructs) adapted from the HBM for this study are illustrated in Figure 1.1 below.

Figure 1.1: Health Belief Model Modified for Health Behavior Intentions



This study examined whether recipients of cardiomyopathy VUS results perceive the threat of cardiomyopathy in ways that potentially impact their intentions to pursue health-related behaviors. Uncertainty is the subjective perception of a lack of knowledge about some aspect of reality that is experienced by individuals that could affect motivation or likelihood to act (Han et al., 2011). Uncertainty experienced by patients has also been defined as the inability to determine the meaning of an illness-related event (Mishel, 1990) such as receiving VUS results. This incompleteness of knowledge or unpredictability associated with VUS is on a continuum. Therefore, the inadequate information concerning disease susceptibility and potential diagnosis is dependent on the level of evidence and pathogenicity probability associated with a VUS.

It was therefore hypothesized that high perceived risk and severity of cardiomyopathy-associated VUS results would prompt recipients to intend to pursue health-related behaviors. Health

behavior intentions included information-seeking, healthcare utilization (frequency of visits to healthcare providers, medical tests - ECG, ECHO, MRI, stress test, additional genetic tests), lifestyle modifications (diet, exercise) since receiving result, and communication of result with valued others.

If a person perceives a threat to a condition such as cardiomyopathy, whether this perception leads to any action will be influenced by the person's beliefs regarding perceived benefits associated with the information received, and the conviction that he/she can execute the health behavior required to reduce the threat (self-efficacy). Cues to action were not assessed in this study.

Significance of the Study

Reclassification of VUS into higher (likely pathogenic or pathogenic) or lower (likely benign or benign)-risk categories remains an important endeavor in clinical genetics as the rapidly changing knowledge can alter these assessments. The American College of Medical Genetics and Genomics (ACMG) guidelines for interpretation of sequence variants recommend pathogenicity probability of >99% for a variant to be classified as pathogenic; <99% to >90% certainty for likely pathogenic; 5% to 90% for VUS; 0.1% to <5% for likely benign; and <0.1% for benign classifications (Richards et al., 2015). When clinical laboratories classify variants as pathogenic, interpreting clinicians are likely to use that designation to alter patient treatment and surveillance (Henderson et al., 2014) or end surveillance in a genotype-negative family member. The wide range of pathogenic probabilities of VUS variants make it challenging for interpreting clinicians to ascribe concrete interpretations and clinical recommendations to VUS results. This study examines the impact of returning VUS results on participants' health behavior intentions.

Next generation sequencing provides the opportunity to uncover potentially causal monogenic mutations with significant risk to the individual concerned. Analytical methods to identify clinically important secondary gene variants from exome sequence data need to be designed and empirically validated alongside an approach for the return of results. Research participants are eager to learn about genomic sequencing results, especially those with potential clinical or personal utility (Graves et al., 2014; Bollinger, Scott, Dvoskin, & Kaufman, 2012; Murphy et al., 2008; Kaufman, Murphy, Scott, & Hudson, 2008). To meet the challenges of returning unexpected and uncertain results to research participants and patients in the near future, it will be important to understand the potential effects of disclosure of uncertain genetic results on participants' risk and severity perceptions and how the results inform behaviors. The ability to convey unexpected and/or uncertain genetic results to research participants and clinically sequenced patients will benefit from an understanding of these issues.

There is only a small body of literature on uncertain genetic test results; most studies are related to receipt of inconclusive results instead of VUS in hereditary breast and ovarian cancer (HBOC). Assessment of the impact of VUS results disclosure across more genetic conditions, other than HBOC, is important for assessing recipient experiences in other disorders as future disclosure guidelines are developed. Understanding the expected benefits and value of returning VUS results to participants not only inform the ongoing debate on the minimum results investigators have an obligation to return, it strengthens the social and moral contract between genetic researchers and participants (Facio et al., 2013). Disclosure of VUS results to actual participants will yield more valid associations and conclusions compared with previous studies of hypothetical VUS recipients.

This study describes the impact of VUS results on factors (susceptibility, severity, benefits, and self-efficacy) that predict participants' health behaviors intentions according to the HBM. The HBM is

one of the most widely used conceptual frameworks in health behavior research and interventions. Understanding the various constructs that predict health behaviors following disclosure of genetic information could help researchers and clinicians personalize approaches to returning genetic incidental findings.

The use of the genomic-first approach in ClinSeq® may identify asymptomatic research participants with genetic susceptibility to cardiomyopathy that may not have been identified using a family history-based approach or standard genetic pathogenicity criteria. The availability and access to phenotypic data in the ClinSeq® cohort provides an opportunity to perform iterative clinical research to assess and monitor the pathogenicity of these VUS.

Finally, although the intentions of participants to receive results associated with treatable or preventable conditions can be rationalized, the impact of uncertain genetic data on participants' health behaviors needs further exploration. The perceptions of research participants on how personal genetic data are processed, analyzed, interpreted, and returned has significant impact on the value attached to the returned results, their willingness to participate in future research studies, regret associated with decision to learn genetic information, and health-promoting behaviors that result from learning results.

Innovation

Next generation sequencing allows many genes other than those under investigation to be evaluated. Determination of the clinical significance of secondary gene variants from asymptomatic individuals provides an alternative approach to estimating disease risk, prevention, and management. The use of gene variant-to-phenotype (genomics-first) approach in this study changes the focus from

the usual diagnosis and treatment of disease (disease-first approach) to early identification, effective monitoring, and prevention aimed at disease modification in otherwise healthy individuals with identifiable genetic susceptibility. Using the genomics-first approach, this study identified cardiomyopathy susceptibility variants of uncertain significance from a set of exome sequence data. Again, it is important to emphasize that these individuals were not sequenced because they had a personal or family history of cardiomyopathy; therefore, such results are considered incidental or secondary. Exploration of whether the limitations surrounding the interpretation of VUS will affect participants' health-related behaviors should inform the debate on what ought to be disclosed by researchers and how to involve participants in the evolution of genetic technology and interpretation in order to temper some of their expectations.

Review of Relevant Concepts

Uncertainty surrounding health risks can affect illness perceptions and change the way individuals interpret susceptibility to disease. There is limited information about the way people process and react to VUS results, and most of the available data come from hypothetical studies of VUS recipients. The concept of uncertainty is a significant factor in how a recipient will respond to VUS result. Uncertain medical information has been related to patients' perceptions of illness and coping (Mishel, 1990; Mccormick, 2002). This review explores the concept of uncertainty in healthcare, genetic testing and VUS, and personal utility of genetic information, followed by a description of cardiomyopathy susceptibility.

Uncertainty

Although much has been written about uncertainty by researchers from various disciplines, rarely has the term itself been explicitly defined. Han and colleagues define uncertainty as a subjective, cognitive experience of people – a state of mind rather than a feature of the objective world – that appears to be lack of or incomplete knowledge about some aspect of reality (Han, Klein, & Arora, 2011). Uncertainty exists when situations or outcomes are ambiguous, complex, or probabilistic and might also be experienced when a person assesses the probability of an outcome, such as disease risk (Luther & Crandall, 2011). There are various conceptualizations of uncertainty and ambiguity. Grenier and colleagues define uncertainty as the emotional state provoked by aversion to future-oriented ambiguous stimuli, while ambiguity is the static component present where a situation is characterized by equivocal features (Grenier, Barrette, & Ladouceur, 2011). The difference is one of time-orientation; ambiguity can be described as an inexactness or double meaning, while uncertainty lacks predictability (Luther & Crandall, 2011). However, ambiguity and uncertainty are used interchangeably in this study. The expectation in healthcare is that specific actions will lead to desirable and attainable outcomes. As shared decision-making by healthcare providers and patients increases, helping patients understand scientific uncertainty will become a valuable competency of both researchers and clinicians (Han et al., 2011). There is a good possibility that healthcare providers do not adequately highlight the likelihood of an uncertain genetic test result during the consent process or prior to performing the test. This could be due to anticipated patient dissatisfaction or lack of specific disclosure guidelines and management for patients that receive uncertain results.

Uncertainty in genetic testing can affect illness representations (an individual's perception and belief about an illness) and have a role in the coping process (Shiloh, 2006). Variations in illness representation can lead to differences in perceived risks and benefits of medical decision-making.

Uncertainty as it relates to illness perception has been described as the inability to determine the meaning of illness-related outcomes, leading to the evaluation that the state of one's illness is vague or unclear (Mishel, 1990). Individuals create their own understanding of illness or disease threat (illness representation) and perceive genetic diseases as less controllable, less preventable, and more threatening than other diseases (Shiloh, 2006; Greene, Richards, Murton, Statham, & Hallowell, 1997; Senior, Marteau, & Weinman, 1999). The present study contributes to understanding how uncertainty related to VUS disclosure affect recipients' illness representation.

Perceived uncertainty occurs when an event cannot be classified or appraised as either dangerous or beneficial. A cognitive structure of the event cannot be formed and the person's ability to appraise the situation is compromised. Management of uncertainty in genetics and genomics is achieved through reclassification of results using additional information collected from testing other at-risk relatives of the proband to determine segregation of the identified variant in the family. Management of uncertainty has also been described as an internal cost-benefit analysis, suggesting that when the negative appraisal of an outcome is great, the error that underestimated the outcome is perceived as more costly (Weber, 1994). Little is known about the degree to which individuals adhere to this framework upon VUS disclosure, and how patients under- or overestimate their risks and consequences in response to receiving VUS results.

Consequences of Uncertainty: Individuals with low tolerance for uncertainty are less adept at coping with genetic testing uncertainty, resulting in increased stress (O'Neill, McBride, Alford, & Kaphingst, 2010) and dissatisfaction with healthcare (Politi, Clark, Ombao, Dizon, & Elwyn, 2011). High levels of uncertainty can also lead to anxiety, helplessness, or depression (Livneh & Antonak, 2005).

Understanding the consequences of uncertainty enhances our ability to explain its effect on behavior and hopefully develop intervention strategies to support recipients of VUS results.

Genetic Testing and VUS

Most of the research examining uncertain or ambiguous genetic testing have been conducted using BRCA 1/2 genes in hereditary breast and ovarian cancer patients and carriers, with a majority of the focus on uninformative or inconclusive test results or a true negative result (Antoniou et al., 2003). An inconclusive result is defined as the absence of a pathogenic variant after testing, while an unclassified result is the presence of a variant, which is unknown to be benign or pathogenic. Uncertainty associated with an inconclusive result plays a role in risk perception, distress, and behavior of the recipient. Although in a genetic result showing a VUS, a sequence variant is actually detected as compared with an inconclusive test result (absence of a mutation), there is some evidence that those who receive VUS results are most similar to those receiving inconclusive result based on comprehension of information, risk perception, and psychological distress post disclosure (van Dijk et al., 2004).

The relationship of VUS results disclosure to distress assumes that the participants correctly understood and interpreted disclosed results as uncertain. Some studies report that individuals with a VUS experienced some anxiety and frustration from lack of concrete answers regarding their disease risk (Vink, G.R., Van Asperen, C.J., Devilee, Breuning, & Bakker, 2003; Petrucelli, Lazebnik, Huelsman, & Lazebnik, 2002) and disclosure of VUS results to individuals with high familial risks for disease evoke more psychological distress by maintaining uncertainty about their genetic status (van Dijk et al., 2005; Vadaparampil, Wey, & Kinney, 2003). A rise in uncertainty after VUS disclosure (compared to pre-disclosure) was noted in a few studies, with participants reporting uncertainty to be an important issue

(Chimera, Brooks, Singletary, & Young, 2002; Rao et al., 2006). However, in other studies comprised of individuals with implicated family history, VUS disclosures did not seem to cause more psychological distress than receiving more certain genetic results (Facio et al., 2011; van Dijk et al., 2004). These studies question the sensitivity of general distress measures used to capture the subtle impact of VUS results on research participants as the contextual meaning of these measures was not always clear, due to the absence of comparison with other relevant stressors and reference groups (Coyne, Kruus, Racioppo, Calzone, & Armstrong, 2003).

There have been contradictory results reported on risk perceptions following disclosure of VUS results to research participants. Some studies of distorted perceptions of uncertain genetic results report that research participants perceive VUS risks as lower likelihood of having the variant, lower risk of developing disease, or the absence of genetic predisposition (Claes et al., 2004; Hallowell et al., 2002; Kelly et al., 2005), while other studies did not report similar conclusions (Dorval et al., 2005). In a study by Vos and colleagues, women who received VUS results for *BRCA1/2* made surgical decisions with rates that overlap those reported for women with pathogenic variants, with perception of risk, not recall of VUS information provided by healthcare provider, related to the decision for medical intervention, including prophylactic surgery (Vos et al., 2008). These findings show that VUS results were perceived less accurately than other genetic results and that there is a need for clarity when discussing uncertainty related to VUS in genetic susceptibility to disease.

Personal Utility of Uncertain Genetic Results

Recommendations for return of genome sequencing results have generally focused on the clinical utility of results to the recipients (Fabsitz et al., 2010; Ravitsky & Wilfond, 2006). Some consensus is developing among researchers regarding the return of certain genetic results based on

preferences of research participants seeking to learn personal information (Kohane & Taylor, 2010). The desired information may not fall under the guidelines of medically actionable research findings but could be valued by research participants in ways that differ from the researcher's clinical utility criteria for disclosing results. Although research participants undergoing genome sequencing generally lack the experience to prepare for the psychological impact of receiving uncertain DNA results of varying medical significance, there is still a role for both clinical and personal utility in the return of results (Ormond et al., 2012).

Cardiomyopathy and Arrhythmia

Cardiomyopathy is a myocardial disorder in which the heart muscle is structurally and functionally abnormal, in the absence of coronary artery disease, hypertension, valvular disease and congenital heart disease sufficient to cause the observed myocardial abnormality (Elliott et al. 2008). Cardiomyopathy is a weakening of the heart muscle that often occur when the heart cannot pump as well as it should. The main types of cardiomyopathy are: dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC). Other types of cardiomyopathy are referred to as unclassified cardiomyopathy. DCM is the most common type of the disease; mostly occurring in adults ages 20-60. Men are more likely than women to have this type of cardiomyopathy. DCM is a common cause of congestive heart failure. The exact prevalence of DCM in the general population is unknown, but it clearly varies with age and geography. Around 30-50% of cases have a familial component, and more than 30 genes have been identified that cause DCM with most inherited in an autosomal dominant pattern (Towbin & Bowles, 2002). Genes implicated in DCM code for a variety of proteins expressed within the cardiomyocyte. Penetrance of DCM is incomplete and age-dependent. HCM is structurally

characterized by left ventricular hypertrophy (LVH), predominantly of the interventricular septum, myocyte disarray, and fibrosis (Hershberger, Cowan, Morales, & Siegfried, 2009). It affects approximately 1/500 individuals in the general population, arising most commonly around puberty, associated primarily with mutations in genes encoding sarcomeric contractile proteins. HCM usually follows an autosomal dominant pattern of inheritance with variable expressivity and age-dependent penetrance. Unlike DCM, mutations in two genes, *MYH7* and *MYBPC3*, account for about 80% of HCM cases when associated with a genetic cause (Richard et al., 2003). ARVC is an uncommon disease characterized by right ventricular fibro-fatty replacement, myocyte loss, and wall thinning. Diagnostic criteria include functional and structural alteration of the right ventricle, ECG depolarization/repolarization changes, and arrhythmia/conduction abnormalities (McKenna et al., 1994). Onset usually occurs during adolescence or young adulthood, with SCD and/or exercise-induced ventricular arrhythmia as presenting features. Involvement of the left ventricle may also be present and can precede right ventricle manifestations or even be the presenting feature. One-third to one-half of ARVC cases are familial, with the pattern of inheritance being autosomal dominant with variable expressivity and reduced penetrance (McKenna et al., 1994). Eight genes have been associated with ARVC, most of which encode cardiac desmosomal proteins that predispose to myocyte damage and fibro-fatty replacement (Sen-Chowdhry, Lowe, Sporton, & McKenna, 2004). Restrictive cardiomyopathy (RCM) tends to mostly affect older adults and is not generally considered a genetic cardiomyopathy. With this disease, the ventricles become stiff and rigid as a result of abnormal scar tissue replacing normal heart tissue. The ventricles are unable to relax normally and fill with blood, and the atria become enlarged. Over time, blood flow in the heart is reduced, leading to complications such as heart failure and arrhythmias (nhlbi.nih.gov/health/health-topics/cm/types.html).

Cardiac arrhythmia is a condition in which the electrical activity of the heart is irregular, either faster or slower than normal. Arrhythmias are produced by perturbations of cardiac impulse initiation and abnormalities of impulse conduction. Arrhythmia can manifest in any chamber of the heart, at any age, and can either be barely perceptible or lead to sudden cardiac death. Arrhythmias may lead to reduced blood flow to the organs of the body leading to reduced ability to exercise, faintness, and in cases where blood flow is too low, death (Luchsinger & Steinberg, 1998). Genes associated with monogenic forms of arrhythmia include *KCNQ1* and *KCNH2* (potassium channel genes with autosomal dominant inheritance pattern, associated with long QT syndrome), *SCN5A* (sodium channel gene associated with Brugada syndrome), and *RYR2* (cardiac ryanodine receptor gene with autosomal dominant inheritance pattern, associated with catecholaminergic polymorphic ventricular tachycardia -CPVT) (Kaab & Schulze-Bahr, 2005). Criteria for diagnostic investigation include a 12-lead ECG at rest and during the arrhythmia, clinical history of paroxysmal and irregular palpitations, ambulatory 24-hour Holter monitoring, teaching patients to perform vagal maneuvers, and use of beta-blockers (Blomstrom-Lundqvist et al., 2003).

Current recommendations for management of genetic cardiomyopathy can be summarized as: obtaining a careful family history of at least 3-4 generations; screening (ECHO, ECG, examination) of at-risk relatives; counseling regarding heritable genetic basis, pattern of inheritance, age of onset, and symptoms and; genetic testing as appropriate (Hershberger, Cowan, Morales, & Siegfried, 2009). These recommendations are straight-forward in cases where the identified variant is supported in the literature and classified as pathogenic, but may be more complex and problematic when applied to cardiomyopathy or arrhythmia-associated VUS. This study examined some of the impact of receiving a

cardiomyopathy-associated VUS could improve our understanding of the consequences of VUS disclosures outside of the context of hereditary breast and ovarian cancer variants.

Association between Disease and Genetic Variants

Association of genetic variants and disease has resulted in the identification of multiple causative genes. Two broadly described types of true association include: Firstly, some subpopulation of individuals may share a common ancestor in whom the variant (marker allele) was present on the same chromosome as the disease mutation and there have been insufficient recombinant events between the disease and variant loci to achieve linkage equilibrium. Secondly, the variant itself may increase susceptibility to the disease, in which case association can be observed in both unrelated and related individuals.

There could be a higher prevalence of a variant and disease within a subpopulation where nonrandom mating produces a spurious association even though the loci are not linked. This is known as population stratification (Curtis, 1997). Approaches to guard against spurious (false positive) associations include the use of cases and controls from the same subgroups, and the use of relatives as controls by using the alleles not transmitted by parents or grandparents to affected subjects as an internal control (Spielman, McGinnis, & Ewens, 1993).

The Five-Category Classification System

A five-category classification system conveys information about the relevance of a variant to clinical practice by grading variants as pathogenic (class V: >99% pathogenicity probability); likely pathogenic (class IV: 99% - >90%); VUS (class III: 90% - 10%); likely benign (class II: <10% - 0.1%); benign (class I: <0.1%). This system places variants in categories based on the level of confidence in the evidence used to establish their causal association with disease in a Mendelian context. This

classification system allows variants to be reclassified as evidence from segregation studies, prediction models, or functional assays become available. It is hoped that using this classification system in clinical and research settings could standardize the transmission of variant information to clinicians, patients, and research participants (Richards et al., 2015; Plon et al., 2008; Plon et al., 2011). The five-category system was developed to express the output of a Bayesian method for assessing variants by combining several independent data types to calculate an integrated posterior probability for variant pathogenicity (Tavtigian et al., 2008). This classification system has been used in various genetic studies (Ng et al., 2013; Johnston et al., 2012; Grandval, Fabre, & Olschwang, 2013; Bonadona et al, 2011) and continues to gain acceptance among genetic researchers.

Variants of Uncertain Significance (VUS)

The interrogation of genes associated with disease using clinical (exome, genome, Sanger) sequencing has detected variants with the full range of pathogenicity, from zero to essentially 100%. A consequence of high-throughput, next generation sequencing has been the identification of increasing number of variants whose association with disease risk is not certain. VUS results are more frequent in minority ethnic populations partly due to underrepresentation in genetic research and underutilization of genetic tests (Noll et al., 2002). Various types of evidence may help characterize variants as pathogenic or benign including familial segregation analysis, population frequency, location of the amino acid substitution, degree of conserved functional domain, prediction algorithms (SIFT, PolyPhen-2, CADD, and Mutation Taster), or functional assays (Richards et al., 2015; Spearman et al., 2008; Thompson, Easton, & Goldgar, 2003).

Currently, many groups and laboratories are cautious in classifying variants as pathogenic unless there is overwhelming epidemiologic evidence of association with disease, therefore most variants are listed as VUS's (Plon et al., 2008). Reclassification of VUS into higher or lower-risk categories will remain an important problem in clinical genetics as an increasing number of VUS are identified using technologically improved high throughput sequencing. Genetic testing for hereditary diseases present problems associated with interpretation in clinical practice. Analysis of the Myriad Genetics Laboratory data for breast cancer susceptibility testing (*BRCA1/2*) showed that a clinician has a similar likelihood of receiving a VUS result as one with a pathogenic mutation, with likelihood of VUS result even higher for individuals from understudied populations as they present greater interpretation challenges due to inadequate available data (Plon et al., 2008; Frank et al., 2002).

To reduce the risk of misinterpretation and misinformation, a classification system for gene variants (such as the five-category system) that recommends uniform reporting by laboratories with the goal of providing defined classification and communication of findings is needed (Richards et al., 2015). Development of a classification system requires consideration of a vast amount of evidence of relevance to the disease process and adherence to standards and recommendations developed by content experts. The likelihood of pathogenicity range for VUS is 10 – 90%. The degree of uncertainty could be significantly altered by additional data favoring pathogenicity, which makes it challenging to provide useful concrete clinical recommendations.

Incidental (Secondary) Findings

Next generation sequencing has the potential to generate other clinically important results separate from the mutation causing the disorder for which the sequencing was performed. These off-

target clinical results that may nonetheless be of medical value or utility are termed incidental or secondary findings. Exome sequencing on 572 ClinSeq participants not ascertained for hereditary breast and ovarian cancer yielded 334 variants of potential clinical importance, with seven participants having pathogenic variants in *BRCA1* or *BRCA2* (Johnston et al., 2012). The potential for next-generation sequencing technology to identify incidental variants and whether these variants should be returned to research participants is a source of controversy (Bredenoord, Kroes, Cuppen, Parker, & van Delden, 2011). There is no consensus on whether researchers and clinicians should disclose incidental findings to participants (Wolf et al., 2008). Some researchers and clinicians favor a restrictive disclosure policy where only life-saving data with strong evidence of benefit are returned to participants, while others favor an intermediate position of qualified disclosure whereby genetic findings should be disclosed if they meet certain predetermined conditions. There is evidence in the literature that research participants are interested in, and expect the return of some genetic results as a condition of enrollment even if nothing could be done with the information (Kauffman, Murphy, Scott, & Hudson, 2008). Interviews examining public perceptions show that a majority of the public believes that research studies should have provisions for the return of results to participants (Bollinger, Scott, Dvoskin, & Kaufman, 2012).

The American College of Medical Genetics and Genomics (ACMG) recommendations favor reporting of incidental findings for all clinical germline exome and genome sequencing except for fetal samples. Based upon available evidence, the ACMG Working Group of experts determined that reporting some incidental findings would likely have medical benefit for the patients and family members of patients undergoing clinical sequencing (Green et al., 2013). The ACMG recommendations

restrict variants to be reported as incidental findings to those that meet criteria for reporting as Pathogenic.

Recognition that clinically important genetic results may be present in massively parallel sequencing (MPS) datasets is prompting researchers to develop approaches to address secondary genetic variants and how these variants should be returned to research participants and patients (Ng et al., 2013; Johnston et al., 2012). In the era of next-generation sequencing, secondary variants are becoming both a predictable risk and benefit of research. This study examined the impact of disclosing genetic VUS results on participants' health behavior intentions.

Dissertation Organization

This dissertation consists of six chapters. **Chapter one** provides a background overview of the study, with attention to the purpose and aims of the dissertation, theoretical framework that guided the study, and review of relevant concepts.

Chapter two (Manuscript One) provides a literature review of the pathogenicity classification of genetic variants for sudden cardiac death-related disorders. Cardiomyopathy is one of the sudden cardiac death related diseases we chose to study. Thirty-two studies met the criteria for inclusion in this literature review. Two (2) studies evaluated evidence of disease-gene association, 9 used segregation analysis to inform their assessment, and 24 used information from *in silico* prediction algorithms. Five (5) studies used functional assays and 3 studies included mutation type and location in classifying variants. We concluded that inconsistencies in variant classification, including variations in evidence use, are a challenge in cardiac genetics. We suggest that the American College of Medical Genetics and Genomics (ACMG) guidelines can help with this challenge. The target audience for this

manuscript will be clinicians and researchers seeking to gain understanding on the nature of evidence used to classify genetic results. The target journal for this manuscript is *PLoS One*.

Chapter three outlines the methodology used to guide this research including study design, study sample, data collection procedures and measures, protection of human subjects, and data analysis plan.

Chapter four (Manuscript two) examined the effect of decisions to learn uncertain genetic information such as VUS on regret following disclosure. Participants in this study were given a choice of learning about their genetic result classified as a VUS. The level of regret associated with their decision was measured 2 weeks post disclosure using the Decision Regret Scale (DRS). Sixty-one (61) respondents were included in this analysis. Sixty-six (66) percent of participants reported no regret associated with their decision, and sub-classification of VUS results into High and Low groups based on a predicted deleteriousness analysis showed no significant correlation with regret post disclosure. The target journal for this manuscript is *Medical Decision Making.*

Chapter five (Manuscript three) reports the major findings from this study. In this exome cohort, perceived risk and perceived severity of cardiomyopathy-associated genetic VUS results were weak predictors of intentions to pursue health related behaviors. Perceived information value was the strongest predictor of health behavior intentions. Self-efficacy had a weak effect on intentions but was not statistically significant. A model including perceived risk, perceived severity, perceived value of information, and self-efficacy explained almost 40% of the variance in health behavior intentions of VUS recipients. Resilience, optimism, and tolerance of uncertainty did not modify the effects of the measures examined in this study on health behavior intentions. The target journal for this manuscript is *Clinical Genetics.*

Chapter six presents a concise summary of the dissertation work and integration of findings. This chapter also includes limitations to the overall study. Findings not reported in chapters 1-5 are presented here. It concludes with a discussion regarding the contribution this research makes to the disclosure of uncertain genetic results to research participants and patients while offering suggestions for further study.

References

Antoniou, A., Pharaoh, P.D., Narod, S., Risch, H.A., Eyfjord, J.E., Hopper, J.L., et al. (2003). Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet*, 72(5), 1117-30.

Bandura, A. (1977). Self-efficacy: toward a unifying theory of behavioral change. *Psychological Review*, 84, 191-215

Biesecker, L.G., Mullikin, J.C., Facio, F.M., et al. (2009). The ClinSeq Project: piloting large-scale genome sequencing for research in genomic medicine. *Genome Res,* 19, 1665-1674.

Blomstrom-Lundqvist, C., Scheinman, M.M., Aliot, E.M., Alpert, J.S., Calkins, H., Camm, A.J., et al. (2003). ACC/AHA/ESC guidelines for the management of patients with supraventricular arrhythmias. *J Am Coll Cardiol,* 42(8), 1494-1526.

Bredenoord, A.L., Kroes, H.Y., Cuppen, E., Parker, M., & van Delden, J.J. (2011). Disclosure of individual genetic data to research participants: The debate reconsidered. *Trends in Genetics,* 27, 41-47.

Bollinger, J.M., Scott, J., Dvoskin, R., & Kaufman, D. (2012). Public preferences regarding the return of individual genetic research results: findings from a qualitative focus group study. *Genet Med,* 14, 451-457.

Bonadona, V., Bonaiti, B., Olschwang, S., Grandjouan, S., Huiart, L., Longy, M., et al. (2011). Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. *JAMA*, 305(22), 2304-10.

Carere, D.A., Kraft, P., Kaphingst, K.A., Roberts, J.S., & Green, R.C. (2016). Consumers report lower confidence in their genetics knowledge following direct-to-consumer personal genomic testing. *Genet Med,* 18(1), 65-72.

Chimera, C., Brooks, K., Singletary, C., & Young, S. (2002). The effects on patients after receiving a result of a variant of uncertain clinical significance for BRCA1 and BRCA2 testing. *Genet Counsel* (Abstract from the Twenty-First Annual Education Conference of the National Society of Genetic Counsellors), 11: 455.

Coyne, J.C., Kruus, L., Racioppo, M., Calzone, K.A., & Armstrong, K. (2003). What do ratings of cancer-specific distress mean among women at high risk of breast and ovarian cancer? *American Journal of Medical Genetics,* 116A, 222-228.

Claes, E., Evers-Kiebooms, G., Boogaerts, A., Decruyenaere, M., Denayer, L., & Legius, E. (2004). Diagnostic genetic testing for hereditary breast and ovarian cancer in cancer patients: women's looking back on the pre-test period and a psychological evaluation. *Genet Test*, 8, 13-21.

Curtis, D. (1997). Use of siblings as controls in case-control association studies. *Annals of Human Genetics,* 61(4), 319-33.

Dorval, M., Gauthier, G., Maunsell, E., Dugas, M.J., Rouleau, I., Chiquette, J., et al. (2005). No evidence of false reassurance among women with an inconclusive BRCA1/2 genetic test result. *Cancer Epidem Biomar,* 14, 2862-2867.

Dugas, M.J., Gosselin, P., Ladouceur, R. (2001). Intolerance of uncertainty and worry: investigating narrow specificity in a non-clinical sample. *Cognitive Therapy and Research,* 25, 551-558.

Dyer, J.G., & McGuiness, T.M. (1996). Resilience: Analysis of the concept. *Archives of Psychiatric Nursing,* 10, 276-282.

Elliott, P., Andersson, B., Arbustini, E., Bilinska, Z., Cecchi, F., Charron, P., et al. (2008). Classification of the cardiomyopathies: a position statement from the European society of cardiology working group on myocardial and pericardial diseases. *European Heart Journal,* 29(2), 270-276.

Elwyn, G., & Miron-Shatz, T. (2010). Deliberatrion before determination: the definition and evaluation of good decision making. *Health Expect,* 13(2), 139-47.

Fabsitz, R.R., McGuire, A., Sharp, R.R., Puggal, M., Beskow, L.M., Biesecker, L.G., et al. (2010). Ethical and practical guidelines for reporting genetic research results to study participants: updated guidelines from a national heart, lung, and blood institute working group. *Circ Cardiovasc Genet,* 3, 574-580.

Facio, F.M., Brooks, S., Loewenstein, J., Green, S., Biesecker, L.G., & Biesecker, B.B. (2011). Motivators for participation in a whole-genome sequencing study: implications for translational genomics research. Eur J Hum Genet, 19(12), 1213-1217.

Facio, F.M., Eidem, H., Fisher, T., Brooks, S. Linn, A., Kaphingst, K.A., et al. (2013). Intentions to receive individual results from whole-genome sequencing among participants in the ClinSeq study. Eur J Hum Genet, 21(3), 261-265.

Ferriere, M., & Van Ness, B. (2012). Return of individual research results and incidental findings in the clinical trials cooperative group setting. *Genet Med,* 14, 411-416.

Frank, T.S., Deffenbaugh, A.M., Reid, J.E., Hulick, M., Ward, B.E., Lingenfelter, B., et al. (2002). Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. *J Clin Oncol,* 20, 1480-1490.

Giltay, E.J., Kamphuis, M.H., Kalmijin, S., Zitman, F.G., Kromhout, D. (2006). Dispositional optimism and the risk of cardiovascular death: The Zutphen Elderly Study. *Archives of Internal Medicine*, 166, 431–436.

Graves, K.D., Sinicrope, P.S., Esplen, M.J., Peterson, S.K., Patten, C.A., Lowery, J., et al. (2014). Communication of genetic test results to family and health-care providers following disclosure of research results. *Genet Med,* 16(4), 294-301.

Grandval, P., Fabre, A.J., & Olschwang, S. (2013). Design of a core classification process for DNA mismatch repair variations of a priori unknown functional significance. *Human Mutation,* 34(6), 920-2.

Green, R.C., Berg, J.S., Grody, W.W., Kalia, S.S., Korf, B.R., Martin, C.L., et al. (2013). ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genetics in Medicine,* 15(7), 565-574.

Greene, J., Richards, M., Murton, F., Statham, H., & Hallowell, N. (1997). Family Communication and Genetic Counseling: The Case of Hereditary Breast and Ovarian Cancer. *Journal of Genetic Counseling,* 6, 45-60.

Grenier, S., Barrette, A.M., & Ladouceur, R. (2011). Intolerance of Uncertainty and Intolerance of Ambiguity: Similarities and Differences. *Personality and Individual Differences,* 39 (3), 393-600.

Hallowell, N., Foster, C., Andern-Jones, A., Eeles, R., Murday, V., & Watson, M. (2002). Genetic testing for women previously diagnosed with breast/ovarian cancer: examining the impact of BRCA1 and BRCA2 mutation searching. *Genet Test*, 6, 79-87.

Han, P.K., Klein, W.M., Arora, N.K. (2011). Varieties of uncertainty in health care: a conceptual taxonomy. *Med Decis Making*, 31(6), 828-38.

Han, P.K., Klein, W.M., Lehman, T., Killam, B., Massett, H., & Freedman, A.N. (2011). Communication of uncertainty regarding individualized cancer risk estimates: effects and influence factors. *Med Decis Making,* 31(2), 354-66.

Henderson, L.B., Applegate, C.D., Wohler, E., Sheridan, M.B., Hoover-Fong, J., & Batista, D.A. (2014). The impact of chromosomal microarray on clinical management: a retrospective analysis. *Genet Med,* 16, 657-664.

Hershberger, R.E., Cowan, J., Morales, A., Siegfried, J.D. (2009). Progress with genetic cardiomyopathies: screening, counseling, and testing in dilated, hypertrophic, and arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circulation: Heart Failure*, 2(3), 253-61.

Janz, N.K., Becker, M.H. (1984). The health belief model: A decade later. *Health Education & Behavior*, 11(1), 1-47.

John, E.M., Miron, A., Gong, G., Phipps, A.I., Felberg, A., Li, F.P., et al. (2007). Prevalence of pathogenic BRCA1 mutation carriers in 5 US racial/ethnic groups. *JAMA,* 298, 2869-2876.

Johnston, J.J., Rubinstein, W.S., Facio, F.M., Ng, D., Singh, L.N., et al. (2012). Secondary variants in individuals undergoing exome sequencing: Screening of 572 individuals identifies

high-penetrance mutations in cancer susceptibility genes. *Am J Human Genetics,* 91, 1-12.

Joseph-Williams, N., Edwards, A., & Elwyn, G. (2011). The importance and complexity of regret in the measurement of 'good' decisions: a systematic review and content analysis of existing assessment instruments. *Health Expect,* 14, 59-83.

Kaab, S., & Schulze-Bahr, E. (2005). Susceptibility genes and modifiers for cardiac arrhythmias. *Cardiovascular Research*, 67(3), 397-413.

Kaufman, D., Murphy, J., Scott, J., & Hudson, K. (2008). Subjects matter: a survey of public opinions about a large genetic cohort study. *Genet Med*, 10(11), 831-839.

Kelly, K., Leventhal, H., Andrykowski, M., Toppmeyer, D., Much, J., Dermody, J., et al. (2005). Using the common-sense model to understand perceived cancer risk in individuals testing for BRCA1/2 mutations. *Psycho-Oncology*, 14, 34-48.

Kohane, I.S., & Taylor, P.L. (2010). Multidimensional results reporting to participants in genomic studies: Getting it right. *Sci Transl Med,* 2, 37cm19.

Kubzansky, L.D., Sparrow, D., Vokonas, P., & Kawachi, I. (2001). Is the glass half empty or half full? A prospective study of optimism and coronary heart disease in the Normative Aging Study. *Psycho-somatic Medicine*, 63, 910–916.

Lindor, N.M., Goldgar, D.E., Tavtigian, S.V., Plon, S.E., Couch, F.J. (2013). BRCA1/2 sequencing variants of uncertain significance: a primer for providers to assist in discussions and in medical management. *Oncologist*, 18(5), 518-524.

Livneh, H., & Antonak, R.F. (2005). Psychosocial Adaptation to Chronic Illness and Disability: A Primer for Counselors. *J Counseling & Development, 83*, 12-21.

Luchsinger, J.A., & Steinberg, J.S. (1998). Resolution of cardiomyopathy after ablation of atrial flutter. *J Am Coll Cardiol*, 32, 205-210.

Luthar, S.S., Cicchetti, D., & Becker, B. (2000). The construct of resilience: A critical evaluation and guidelines for future work. *Child Development,* 71, 543-562.

Luther, V.P., & Crandall, S.J. (2011). Commentary: ambiguity and uncertainty: neglected elements of medical education curricula? *Academic medicine: journal of the Association of American Medical Colleges*, *86*(7), 799-800.

Maron, B.J., Gardin, J.M., Flack, J.M., et al. (1995). Prevalence of hypertrophic cardiomyopathy in a general population of young adults: echocardiographic analysis of 4111 subjects in the CARDIA Study Coronary Artery Risk Development in (Young) Adults. Circulation, 92, 785–9.

Mccormick, K.M. (2002). A Concept Analysis of Uncertainty in Illness. *Journal of Nursing Scholarship*, 127-131.

McKenna, W.J., Thiene, G., Nava, A., Fontaliran, F., Blomstrom-Lundqvist, C., Fontaine, G., et al. (1994). Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Task force of the working group myocardial and pericardial disease of the European society of cardiology and of the scientific council on cardiomyopathies of the international society and federation of cardiology. *Br Heart J,* 71, 215-218.

McGuire, A.L., & Lupinski, J.R. (2010). Personal genome research: what should the participant be told? *Trends in genetics: TIG*, *26*(5), 199-201.

Mishel, M.H. (1990). Uncertainty in Illness Theory. *Journal of Nursing Scholarship*, 256–262.

Murphy, J., Scott, J., Kaufman, D., Geller, G., LeRoy, L., & Hudson, K. (2008). Public Expectations for Return of Results from Large-Cohort Genetic Research. *The American Journal of Bioethics*, 8(11), 36-43.

Nanda, R., Schumm, L.P., Cummings, S., Fackenthal, J.D., Sveen, L., Ademuyiwa, F., et al. (2005). Genetic testing in an ethnically diverse cohort of high-risk women: a comparative analysis of BRCA1 and BRCA2 mutations in American families of European and African ancestry. *JAMA,* 294, 1925-1933.

Ng, D., Johnston, J.J., Teer, J.K., Singh, L.N., Peller, L.C., Wynter, J.S., et al. (2013). Interpreting secondary cardiac disease variants in an exome cohort. *Circulation,* 6(4), 337-46.

Noll, W.W., Reid, J.E., Burbidge, L.A., et al. (2002). Prevalence of BRCA1 and BRCA2: analysis of 10,000 individuals. *J Clin Oncology,* 20, 1480-1490.

O'Neill, S.C., McBride, C.M., Alford, S.H., & Kaphingst, K. (2010). Preferences for genetic and behavioral health information: the impact of risk factors and disease attributions. *Annals of behavioral medicine: a publication of the Society of Behavioral Medicine*, *40*(2), 127-37.

Ormond, K.E., Wheeler, M.T., Hudgins, L., Klein, T.E., Butte, A.J., Altman, R.B., et al. (2012). Challenges in the clinical application of whole-genome sequencing. *Lancet*, 375(9727), 7.

Petrucelli, N., Lazebnik, N., Huelsman, K.M., & Lazebnik, R.S. (2002). Clinical interpretation and recommendations for patients with a variant of uncertain significance in BRCA1 or BRCA2: a survey of genetic counseling practice. *Genet Test,* 6, 107-113.

Plon, S.E., Cooper, H.P., Parks, B., Dhar, S.U., Kelly, P.A., Weinberg, A.D., et al. (2011). Genetic testing and cancer risk management recommendations by physicians for at-risk relatives. *Genet Med*, 13(2), 148-54.

Plon, S.E., Eccles, D.M., Easton, D., Foulkes, W.D., Genuardi, M., Greenblatt, M.S., et al. (2008). Sequence variant classification and reporting: Recommendations for improving the interpretation of cancer susceptibility genetic test results. *Human Mutation*, 29(11), 1282-1291.

Politi, M.C., Clark, M., Ombao, H., Dizon, D., & Elwyn, G. (2011). Communicating uncertainty can lead to less decision satisfaction: a necessary cost of involving patients in shared decision making? *Health expectations: an international journal of public participation in health care and health policy*, *14*(1), 84-91.

Rao, S., Liston, L., Robinson, P., et al. (2006). Patient's perceptions of uncertain variant results in the BRCA genes. In: Presented Abstracts from the Twenty-Fourth Annual Education Conference of the National Society of Genetic Counselors. *J Genet Couns,* 15, 7.

Ravitsky, V., & Wilfond, B.S. (2006). Disclosing individual genetic results to research participants. *Am J Bioethics,* 6, 8-17.

Ready, K., Gutierrez-Barrera, A.M., Amos, C., Meric-Bernstam, F., Lu, K., Hortobagyi, G., et al. (2011). Cancer risk management decisions of women with BRCA1 or BRCA2 variants of uncertain significance. *Breast J*, 17, 210–212.

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., et al. (2015). Standards and guidelines for the inter interpretation of sequence variants: a joint consensus

recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med,* **17**(5), 405-24.

Richard, P., Charron, P., Carrier, L., Ledeuil, C., Cheav, T., Pichereau, C., et al. (2003). Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation*, 107, 2227-2232.

Rosenstock, I.M. (1974). Historical origins of the health belief model. *Health Education & Behavior*, 2, 328-335.

Rosenstock, I. M., Strecher, V. J., & Becker, M. H. (1988). Social learning theory and the health belief model. *Health Education Quarterly*, *15*, 175–183.

Senior, V., Marteau, T.M., & Weinman, J. (1999). Impact of genetic testing on causal models of heart disease and arthritis: An analogue study. *Psychol Health*, 14, 1077– 1088.

Scheier, M.F., Matthews, K.A., Owens, J.F., Schulz, R., Bridges, M.W., Magovern, G.J., & Carver, C.S. (1999). Optimism and re-hospitalization after coronary artery bypass graft surgery. *Archives of Internal Medicine*, 159, 829–835.

Sen-Chowdhry, S., Lowe, M.D., Sporton, S.C., & McKenna, W.J. (2004). Arrhythmogenic right ventricular cardiomyopathy: clinical presentation, diagnosis, and management. *Am J Med,* 117, 685-695.

Shiloh, S. (2006). Illness representations, self-regulation, and genetic counseling: a theoretical review. *Journal of genetic counseling*, *15*(5), 325-37.

Spearman, A.D., Sweet, K., Zhou, X.P., McLennan, J., Couch, F.J., & Toland, A.E. (2008). Clinical applicable models to characterize BRCA1 and BRCA 2 variants of uncertain significance. *Journal of Clinical Oncology,* 26(33), 5393-5400.

Spielman, R.S., McGinnis, R.E., & Ewens, W.J. (1993). Transmission disequilibrium test for linkage disequilibrium: the insulin gene region and insulin dependent diabetes mellitus (IDDM). *Am J Hum. Genet,* 52, 506-516.

Stacey, D., Legare, F., Col, N.F., Bennett, C.L., Barry, M.J., Eden, K.B., et al. (2014). Decision aids for people facing health treatment or screening decisions. *Cochrane Database Syst Rev,* Jan 28, (1): CD001431.

Szanton, S.L., & Gill, J.M. (2010). Facilitating resilience using a society-to-cells framework: A theory of nursing essentials applied to research and practice. *Advances in Nursing Science,* 33(4), 329-343.

Tavtigian, S.V., Greenblatt, M.S., Lesueur, F., Byrnes, G.B., for the IARC Unclassified Genetic Variants Working Group. (2008). In silico analysis of missense substitutions using sequence-alignment based methods. *Human Mutation*, 29(11), 1327-1336.

Thompson, D., Easton, D.F., & Goldgar, D.E. (2003). A full-likelihood method for the evaluation of causality of sequence variants from family data. *Am J Hum Genet,* 73, 652-655.

Towbin, J.A., & Bowles, N.E. (2002). The failing heart. *Nature*, 415(6868), 227-233.

van Dijk, S., van Asperen, C.J., Jacobi, C.E., Vink, G.R., Tibben, A., Breuning, M.H., et al. (2004). Variants of uncertain clinical significance as a result of BRCA 1 /2 testing: impact of an ambiguous breast cancer risk message. *Genet Test,* 8, 235-239.

van Dijk, S., Otten, W., Timmermans, D.R.M., van Asperen C.J., Meijers-Heijboer, H., Tibben, A., et al. (2005). What's the message? Interpretation of an uninformative BRCA1/2 test result for women at risk of familial breast cancer. *Genet Med,* 7, 239-245.

Vadaparampil, S.T., Wey, J.P., & Kinney, A.Y. (2003). Psychosocial aspects of genetic counseling and testing. *Sem Oncol Nurs,* 20, 186-195.

Vink, G.R., Van Asperen, C.J., Devilee, P., Breuning, M.H., & Bakker, E. (2003). Nationwide survey about the policy of testing and counseling of unclassified variants in BRCA1 and BRCA2. *Fam Cancer,* 2, 228.

Vink, G.R., van Asperen, C.J., Devilee, P., Breuning, M.H., & Bakker, E. (2005). Unclassified variants in disease-causing genes: nonuniformity of genetic testing and counselling, a proposal for guidelines. *EJHG*, *13*(5), 525-7.

Vos, J., Otten, W., Van Asperen, C., Jansen, A., Menko, F., & Tibben, A. (2008). The counsellees' view of an unclassified variant in BRCA1/2: recall, interpretation, and impact on life. *Psycho-Oncology*, *17*(8), 822–830.

Wade, C.H., Shiloh, S., Woolford, S.W. et al. (2011). Modeling decisions to undergo genetic testing for susceptibility to common health conditions: an ancillary study of the Multiplex Initiative. *Psychol Health,* 27, 430-444.

Weber, E.U. (1994). From subjective probabilities to decision weights: The effect of asymmetric loss functions on the evaluation of uncertain outcomes and events. *Psychological Bulletin*, *115*(2), 228–242.

Wolf, S.M., Crock, B.N., Van Ness, B., Lawrenz, F., Kahn, J.P. Beskow, L.M., et al. (2012) Managing incidental findings and research results in genomic research involving biobanks and archived data sets. *Genet Med,* 14, 361-384.

Wolf, S.M., Lawrenz, F.P., Nelson, C.A., Kahn, J.P., Cho, M.K., Clayton, E.W., et al. (2008). Managing incidental findings in human subjects research: Analysis and recommendations. *J Law Med Ethics*, 36, 219-248, 211.

Zeelenberg, M., & Pieters, R. (2007). A theory of regret regulation 1.0. *Journal of Consumer Psychology*, 17, 3-18

CHAPTER 2: MANUSCRIPT ONE (REVIEW PAPER)

Genetic Variant Classification in Sudden Cardiac Death Related Disorders

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ABSTRACT

Background: There is a broad and heterogeneous range of genetic evidence used to assess the pathogenicity of gene variants. Understanding the variation in the types of evidence that are in current use is critical for cardiac genetics.

Objectives: To review genetic variant classification approaches in genes associated with SCD-related disorders and compare them to recent American College of Medical Genetics and Genomics (ACMG) recommendations for classification and interpretation of sequence variants.

Data Sources: PubMed/Medline (2008-2016) and Embase (2008–2016). Retrieved articles were checked for additional publications.

Selection Criteria and Data Extraction: Studies reporting variant classification process in genes associated with SCD-related disorders. Articles with no clear mention of classification processes and non-English papers were excluded.

Results: The 32 publications selected for this review employed multiple types of genetic evidence for variant classification. Two (2) studies evaluated evidence of disease-gene association. Nine (9) used segregation studies to inform their assessment. Twenty-four (24) used in silico prediction algorithms. Three (3) studies included mutation type and location, and 5 studies used functional assays in classifying variants.

Conclusions: Inconsistencies in variant classification, including variations in evidence use, are a challenge in cardiac genetics. We suggest that the ACMG guidelines can help with this challenge.

INTRODUCTION

Death from cardiac causes within 24 hours from onset of symptoms is termed sudden cardiac death (SCD) (Adabag, Luepker, Roger, & Gersh, 2010), which accounts for about 20% of mortality in the industrialized world (de Vreede-Swagemakers et al., 1997). Clinical ascertainment of at-risk indiviudals prior to SCD remains a challenge. Coronary artery disease is the main cause of SCD in older adults; however, in individuals less than age 40, SCD can also be caused by inherited arrhythmogenic and connective tissue disorders (Eckart et al., 2011; Hoffmann et al., 2013). Inherited arrhythmogenic disorders comprise two groups: channelopathies and cardiomyopathies. Inherited connective tissue disorders associated with SCD include conditions that predispose to aortic and arterial aneurysms and dissection such as Marfan syndrome, Loeys-Dietz syndrome (LDS), Ehlers-Danlos syndrome (EDS), and thoracic aortic aneurysm and dissection (TAAD).

Current SCD risk stratification criteria such as family history, unexplained syncope, New York Heart Association (NYHA) classifications, depressed left ventricular ejection fraction (Gradman et al., 1989; Pezawas et al., 2014), and electro- and echocardiographic measures outside of established limits (Grimm, Christ, Bach, Muller, & Maisch, 2003) are imprecise and lack sufficient sensitivity and specificity to reliably predict SCD.

Given that over half of SCD victims did not have recognized heart disease at their initial event (Myerburg & Junttila, 2012), molecular genetic testing can potentially contribute to effective risk stratification and management of predisposed individuals. The recent joint consensus statement by the Heart Rhythm Society and the European Heart Rhythm Association on the state of predictive genetic testing and counseling for the cardiomyopathies and

channelopathies (Ackerman et al., 2011) argues for genetic screening to prevent SCD. The detection of pathogenic variants in genes associated with SCD could be used to predict susceptibility to SCD in apparently healthy individuals (Priori et al., 2003). However, correctly determining the pathogenicity of gene variants is challenging. The ACMG, Association for Molecular Pathology (AMP), and College of American Pathologists' standards and guidelines for classification and interpretation of variants provide general recommendations for interpreting variants from clinical genetic testing (Richards et al., 2015). Some clinical and research laboratories are beginning to adopt these recommendations (Wooderchak-Donahue et al., 2015; Ziganshin et al., 2015; Koen et al., 2015), while others have developed their own approaches to evaluate the pathogenicity of variants (e.g., Hertz et al., 2015). The focus of this review is to examine approaches to classification of variants in genes associated with SCD.

METHODS

Search methods and study selection

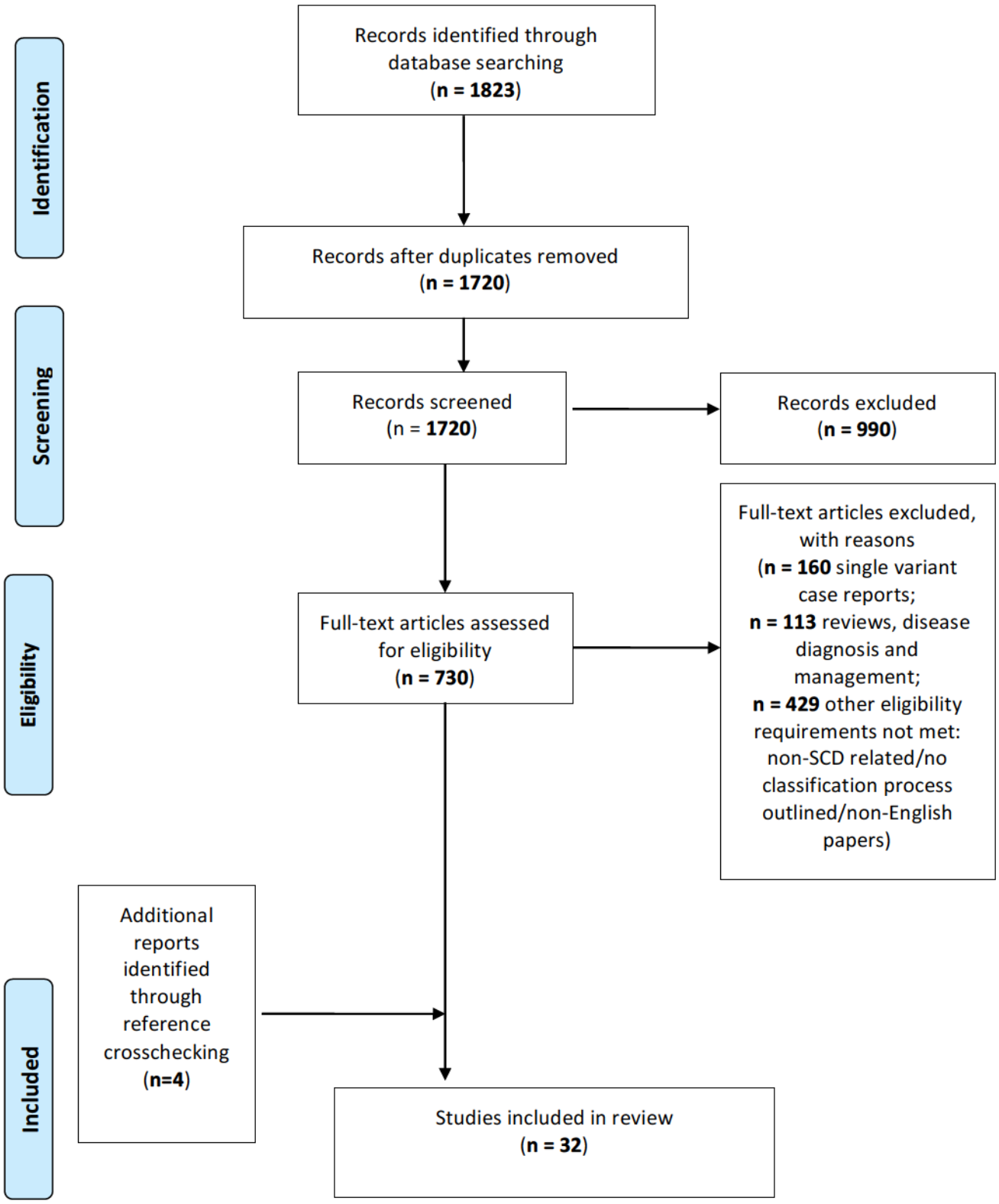
We searched PubMed and Embase (2008-2016) using the search terms "Death, Sudden, Cardiac" and "Genetic Variation" and "Sequence Analysis, DNA" or "High-Throughput Nucleotide Sequencing" or "Mutation, Missense" combined with "Cardiomyopathies" or "Channelopathies" or "Marfan syndrome".

Duplicates and papers not published in English were eliminated, and references from retrieved articles were manually searched for relevant studies. All abstracts were screened and final selection made by reading full articles. Eligible studies were those that reported variant classification processes in genes associated with SCD-related disorders.

RESULTS

The search identified 347 articles in PubMed and 1,476 results (including abstracts and posters) in Embase. Articles focused on challenges regarding classification and management of variants, single variant case reports, posters, editorials, and studies with no outline or mention of the classification process used, were excluded from this review. Twenty-eight (28) articles met these criteria. Crosschecking references from the 28 articles identified four (4) additional articles. The selection and filtering process is summarized in Figure 2.1.

Figure 2.1: Flow diagram for systematic review



The 32 studies selected for this review are described in Table 2.1. These studies analyzed and classified variants in genes associated with SCD related disorders.

Three (3) studies reported on variants in a single gene (Akilzhanova et al, 2014; Hershberger et al., 2009; Kapplinger et al., 2015), and 29 articles evaluated variants in multiple genes (2 to 204) associated with SCD.

Table 2.1: Data Extraction Table for SCD-Related Genes

Author, Year	Diagnostic (D) vs Screening (S)	N (cases)	SCD Related Disease	Gene(s)	Variant Classification System	Criteria for VUS Classification	Comments in paper
Akilzhanova et al., 2014	D	35	CVPT	*RYR2*	Frequency of variant in 1000 Genomes and ESP, and 5 prediction tools. Missense variant classified as possibly damaging when at least three of five prediction tools predicted the variant as damaging and when absent in three queried databases.	Variant with ≤3 "damaging" predictions but at least one positive entry in one of the three databases	Combining sets of prediction scores and reference databases appeared fundamental to predict the pathogenic potential of rare and novel missense variants in populations where genotype data are rare
Bagnall et al., 2014	D	50 Autopsy samples	Sudden Unexplained Deaths	Genes associated with channelopathies and cardiomyopathies.	To identify putatively pathogenic variants, we retained alleles present at <0.1% frequency in any ethnic subgroup from more than 8000 publicly available exomes from the 1000 Genomes Project and in 6500 control exomes from the NHLBI ESP. Variants were		Our preliminary findings suggest that an exome sequencing–based molecular autopsy is a useful strategy as part of the investigation of SUD cases, with many advantages over the gene-by-gene approach of direct Sanger sequencing. Exome sequencing is also useful when clinical and family histories are not available to guide postmortem genetic testing

					excluded if they occurred in >10% of our in-house exome data from 97 individuals with diverse cardiac pathologies (i.e., likely false positives).		
Campuzano et al., 2014	D	1	LQTS	55 SCD-related genes used for detection of sequence and copy number variants. ***TTN*** and ***KCNQ1*** revealed deletion and novel variant respectively.	Allelic frequency, ***In silico*** models, family co-segregation of variant with affected relatives	Novel variant in disease-associated genes in affected individuals	The role of genetic VUS in causing disease should be taken with great caution if family segregation is not available
Campens et al., 2015	S	264	Heritable Thoracic Aortic Disorders (H-TAD)	***FBN1, TGFBR1/2, TGFB2, SMAD3, ACTA2 and COL3A1***	The following information was obtained for each non-synonymous variant to determine pathogenicity: (1) the outcome of missense predictions tools (SIFT, PolyPhen2, Grantham Distance, Align GVGD, Mutation Taster), that are based on sequence homology, the physicochemical similarity between the alternate amino acids, effect of an amino acid	When data remained inconclusive, the variant was classified as a variant of unknown significance (VUS).	Most of the ***FBN1*** mutations found in our cohort were substitutions of a highly conserved cysteine residue involving a cb-EGF-like domain, which is the most common pathogenic ***FBN1*** mutation type. In most cases, interpretation of the functional consequence of typical mutations in ***FBN1*** (cysteine missense mutations) and in ***COL3A1*** (glycine missense mutations) is clear-cut. However, a significant proportion of variants, named VUS, consists of 'atypical' missense changes or potential splice-site changes of uncertain biologic or clinical relevance. The challenging issue remains how to use these data in clinical practice.

					substitution on the structure and function of a protein and conservation level of the amino acid residue amongst species, in case of mutations predicted to result in an amino acid substitution; (2) frequency of the variant in control alleles; (3) literature research including relevant phenotypic information, segregation studies and functional data. Variant segregation analysis was performed if DNA samples of affected and non-affected family members were available. For a variant possibly affecting a splice site the splicing effect at cDNA level was verified if cDNA of the patient was available. Variants were considered as disease causing		

					when there was convincing evidence from the above mentioned criteria about their pathogenicity. In most cases, a combination of different independent variant characteristics, supported the final decision.		
Chung et al., 2009	S	65	Marfan Syndrome, Loeys-Dietz syndrome	***FBN1, TGFBR2***	The criteria adopted to predict whether a novel mutation is causative included: involvement of (a) highly conserved cysteine residues of the cbEGF modules, (b) the highly conserved residues of the calcium-binding motif D/N-x-D/N-E/Q-Xm-D/N*-Xn-Y/F of the cbEGF modules, (c) nonsense mutations, (d) frameshift mutations, and (e) canonical splice sites. For novel mutations not matching these criteria, their potential effects	In silico analysis was used to classify novel mutations of unknown significance.	Functional studies of the protein affected by potential causative mutations are the definitive means to assign causation. However, other tools, such as in silico prediction and accurate selection of orthologs, paralogs and equivalent domains in the protein or related ones with repeating units, can be useful in assessing sequence and structural variation.

					on protein function were analyzed based on the degree of evolutionary conservation of the particular positions in the orthologs and paralogs of FBN1, and in other similar domains in the same protein.		
Das et al., 2014	D	136	HCM	***MYBPC3; MYH7; TNNI3; TNNT2; TPM; MYL2; MYL3; ACTC; ACTN2; TCAP***	Variants reassessed into three categories: Pathogenic, VUS, and Benign. A panel of four experts performed an independent review of all variants to evaluate pathogenicity classification.	VUS were those in which the effect on the protein function was not clear and disease-risk association with HCM was not known.	On the basis of the growth in available human genome data, coupled with increasing knowledge of disease mechanisms, and availability of newer in silico prediction programs, reevaluation of genetic findings over the last decade in clinical settings is of importance
D'Argenio et al., 2014	D	3 unrelated probands	HCM	202 cardiomyopathy-related genes	A frequency filter was used to exclude variants that are either the most frequent allele in populations close to the population of our three subjects (southern Italian), or present with a frequency too high to be consistent with pathogenicity within these or other populations. Variants in which		Although the bioinformatic tools we used eliminated variants that are most likely bereft of clinical significance, the real pathogenic role of a given variant can be determined only with functional studies and/or family segregation analysis to evaluate genotype-phenotype correlations within each family. The variants we identified in the present work should be classified as possibly pathogenic because they fulfill at least one of the following conditions: i) previous findings showing that they are disease-causing (four of them); ii) their presence on genes known to cause HCM (two); and iii) the presence of stop codons indicating a truncated protein (one). Further procedures are of course necessary to confirm the causative origin of mutations to better rationalize the genotype-phenotype relationship within each family and to better understand the molecular basis of the alteration induced by each mutational event.

					the alternative allele frequency was greater than 0.05 in the European super population were filtered out to produce the final set. This multistep selection/annotation procedure produces a prioritized short list of well-annotated variants that may be used to search for pathogenetic changes related to HCM onset and/or clinical features		
Di Resta et al., 2015	S	91 SCN5A-negative BrS patients	Brugada syndrome	158 genes associated with arrhythmic and cardiac defects	Variants defined as 'rare', if they were previously unreported (novel) in the analysed databases ExAC, ESP and dbSNP138, or with an MAF <0.1% in the European population according to the ExAC database. The functional rare variants obtained were also checked in the HGMD database. To determine the likelihood of disease		Functional experiments and segregation studies will be required to clarify the mechanism of action of candidate genes in cardiac electrophysiology and confirm their possible contribution to BrS pathogenesis.

					association, variants were classified in three distinct categories: HGMD mutations (reported in HGMD database); likely mutations (truncating variants: frameshift insertions/deletions, nonsense, splicing); potential mutations (missense, in frame insertions/deletions).		
Farrugia et al., 2015	D	16 autopsy negative cases	Cardiac channelopathies	23 channelopathy-associated genes	Putatively pathogenic variants were determined when all of the following criteria were met together : (i) a minor allele frequency (MAF) in dbSNP (www.ncbi.nlm.nih.gov/SNP) and in the Exome sequencing project (ESP) database with a value under 0.01 (ii) the presence of the variant in less than three samples from our cohort, since previous		Although in silico prediction can be useful to support pathogenicity, the results of this study should be cautiously interpreted. Segregation studies and therefore available family members are a crucial point to understand the possible pathogenicity of each variant classified as “likely pathogenic”. However, in our study no family members could be contacted or wished to be tested and there could not be any follow up.

					mutational studies showed that no highly recurrent mutation was identified in the targeted genes;(iii) the presence of the variant in both forward and reverse reads with a comparable variant frequency in reads of both directions. A variant was classified as “likely pathogenic” when the prediction from either PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) or SIFT (http://sift.jcvi.org/) indicated an effect on the protein, when a high level of conservation in distantly related species was observed or when the pathogenicity of the described variant had already been established by functional testing and when the associated disease or phenotype resulting from the functional change was relevant to	

					the specific clinical condition present in the individuals under study.		
Garcia-Pavia et al., 2011	D, S	165 (probands + relatives)	DCM	***PKP2; DSP; DSC2; DSG2; JUP***	Patients classified as carriers of pathogenic mutations if: they had a genetic variant that was reported in the ARVC database as pathogenic, a novel sequence variant not found in controls that predicts a premature truncation, frameshift or abnormal splicing, a novel missense mutation that affects a conserved amino acid residue and co-segregated with disease on familial evaluation, or a variant classified in the international database as a variant of unknown effect which co-segregated with disease on subsequent family screening. The likelihood of pathogenic effect of missense sequence variants in desmosomal	Variants classified in the international ARVC database as variants of unknown effect or with novel missense mutations not found in controls without corroborative family screening data.	As it is possible that some genetic variants merely increase susceptibility to disease or modify the response to other genetic, epigenetic or environmental factors, testing for desmosomal protein gene mutations should always be performed in conjunction with careful clinical phenotyping and familial evaluation

					genes was determined by four in silico prediction methods		
Golbus et al., 2014	D	11 unrelated subjects	Cardiomyopathy	204 myopathy-associated genes	Variants were analyzed using PolyPhen-2 (PP2), SIFT, PhastCons, GERP, Panther, and ConSeq. Frequency was assessed using three publically available databases: The March, 2012 Integrated Phase 1 release of the 1000Genomes Project, the NHLBI Exome Sequencing Project, and dbSNP 135/136. A minor allele frequency (MAF) of ≤0.01 was used to restrict variants.		These pilot data demonstrate that ~30-40× coverage whole genome sequencing combined with targeted analysis is feasible and sensitive to identify rare variants in cardiomyopathy-associated genes. Missense variants in *TTN* were not considered because these variants are highly prevalent in the general population and vastly exceed the frequency of cardiomyopathy, making them difficult to interpret at this time
Gregers Winkel et al., 2015	D	66	LQTS, BrS; CCD; A Fib; DCM	***SCN5A; SCN1B; SCN2B; SCN3B; SCN4B; GPD1L; SNTA1; CAV3***	To determine whether a missense mutation is likely pathogenic, it requires statistically significant electrophysiological changes in one or several of the most critical in vitro parameters (including primarily current amplitude [in	Missense mutations not fulfilling the noted criteria would be considered as VUS.	Even though the changes in the sodium current suggest a pathogenic role, we consider some variants to be VUS owing to overall results of risk prediction.

					particular sustained] and inactivation kinetics), an allele frequency in the ExAC database below 0.5%, and at least 2 of the following: (1) predicted damaging by SIFT, (2) predicted damaging by PolyPhen-2, (3) conserved through species, or (4) a Grantham score of at least 100.		
Groeneweg et al., 2014	D	9	ARVC	***PKP2; DSP; JUP; DSG2; DSC2; TMEM43; PLN***	The pathogenic potential of the splice site variants was assessed using ***in silico*** prediction analyses of (1) the maximum entropy model (MaxEntScan), (2) the Human Splicing Finder (HSF), (3) NNSPLICE, (4) GeneSplicer, and (5) SpliceSiteFinder-like. Variant minor allele frequency (MAF) was assessed in large control databases. The functional effects of potential splice site variants demonstrated by	VUS's analyzed for their spliceogenic potential using ***in silico*** prediction algorithms and ***in vitro*** mRNA analyses.	***In vitro*** splicing analysis is important for ARVD/C diagnosis and for subsequent patient care and risk management, particularly in exonic ARVD/C splice site variants.

					experimental mRNA analysis.		
Giudicessi et al., 2012	D	388	LQTS	***KCNQ1; KCNH2***	Synergistic use of 4 predictive tools (Conservation, Grantham, SIFT, PolyPhen-2) to distinguish pathogenic from benign non-synonymous SNVs	Not addressed	If sufficiently predictive, the use of ***In silico*** algorithms represents an attractive methodology to enhance the classification of problematic missense variants given the speed and low overhead required to conduct such analyses.
Hertz et al., 2016	D	72	ARVC; BrS; CPVT; DCM; FAF; HCM; LQTS; LVNC; PFVF	100 genes previously associated with cardio-myopathies and channelo-pathies	Variants evaluated and classified independently by two MD's as: likely; unknown or; unlikely to have functional effects. Evidence for pathogenicity included null variants (nonsense, frameshift, near splice sites, initiation codon), known disease-causing amino acid change or residue, functional studies, preva- lence of the variants in affected individuals with the asso- ciated disease, location in exon and/or functional domain with known disease-causing variants, assumed de novo, co-segregation studies and a minor-allele frequency (MAF) below the disease prevalence.	Based on a scoring scheme based on in silico analysis, all variants were sub-classified as VUS 0, 1, 2 or 3 with an increasing prognostic severity	The study shows the potential of genetic analysis of a large number of genes associated with cardiac diseases in forensic med- icine with the use of NGS, as approximately one third of the investigated cases had variants likely to have functional effects.

Hershberger et al., 2009	D	313 probands	DCM	***TNNT2***	clinical and pedigree data for troponin T mutations in these families were supplemented with functional studies with mutant troponin T proteins reconstituted into porcine cardiac myocytes. This approach yielded highly informative calcium sensitivity and maximal force response data that was helpful to augment molecular genetic data for the identified novel mutations.		A key issue in cardiovascular genetic medicine is determining if a putative mutation in a gene known to be associated with dilated cardiomyopathy (DCM) is indeed causative of disease. The combination of clinical, pedigree, molecular genetic and functional data strengthen the interpretation of ***TNNT2*** mutations in DCM.
Hofman et al., 2013	D, S	6944	LQTS; CPVT; BrS; HCM; DCM; LVNC; ARVC (not included in analyses); Others (SQTS, CCD, familial A-Fib, Carney complex, DPP6 associated V-Fib, mitochondrial disease, premature arteriosclerosis)	***KCNQ1; SCN5A; MYBPC3; MYH7; LMNA; PLN; DPP6***	DNA variants classified into 5 groups: nonpathogenic; VUS type 1, 2, 3; or pathogenic mutation, depending on outcome (score) of prediction (in silico) tests. Cosegregation or functional analysis is needed to classify a variant as pathogenic.	VUS1: unlikely to be pathogenic; VUS2: uncertain; VUS3: likely to be pathogenic. In relatives of probands with VUS2 and VUS3, predictive DNA testing was combined with cardiological investigation.	The number of pathogenic variants, VUS3 and VUS2, defines the yield of DNA testing in each disease.

Jordan et al., 2011	D	229	HCM	***MYH7; TNNT2; TPM1; TNNI3; MYBPC3; MYL2***	Manual classification system of variants into five categories.	Insufficient evidence to classify a variant, or evidence is conflicting	VUS not included in development and validation of automated variant pathogenicity prediction method
Kapa et al., 2009	D	388	LQTS	***KCNQ1; KCNH2; SCN5A***	Criteria for pathogenicity probability (estimated predictive value, EPV) based on ethnicity, mutation type and mutation location	Novel mutations in low-EPV regions should be viewed as VUS and prompt further investigation to clarify the likelihood of disease causation.	Given that a timely functional assay does not exist to assess whether a particular VUS can be upgraded or downgraded, further comparative genomic investigations are needed to improve the accuracy of LQTS genetic test.
Kapplinger et al., 2015	D	4999	LQTS; BrS	***SCN5A***	The identified nsSNVs were overlaid on the linear protein topology of $Na_v1.5$. The linear protein topology was annotated to define functionally important domains and structural regions within the cardiac sodium channel. Seven in silico tools were used to assign pathogenic/benign status to nsSNVs from 2888 long-QT syndrome cases, 2111 Brugada syndrome cases. In addition, the in silico tools were	Those rare SCN5A nsSNVs that reside outside of the BrS-derived nsSNVs transmembrane spanning region and those LQTS-derived nsSNVs localizing outside the S3–S5+S6 subdomains or the DIII/DIV IDL specified regions remain ambiguously	The use of a composite score that relies on 7 distinct in silico algorithms may enable rare SCN5A nsSNVs localizing outside of these high probability regions to be upgraded to possibly pathogenic when >4 in silico tools point toward pathogenicity or downgraded further when <4 of the tools do so.

					assessed for their ability to correlate with cellular electrophysiology studies. The use of the composite score allowed for enhanced interpretation for nsSNVs outside of the topological regions that intrinsically had a high probability of pathogenicity	classified as a VUS and require additional cosegregation or functional data to upgrade their probability of pathogenicity at this time.	
Lopes et al., 2015	D	874	HCM	***ACTC1; MYBPC3; MYH7; MYL2; MYL3; TNNI3; TNNT2; TPM1***	Candidate variants defined using MAF ≤0.2%, reports in published literature, and ***In silico*** predicted functional effect	Not addressed	A modifier effect of non-sarcomere gene variants on the influence of sarcomeric variations on HCM phenotype is demonstrated.
Ng et al., 2013	S	870 participants not selected for arrhythmia or cardiomyopathy	Arrhythmia, Cardiomyopathy	22 cardiac arrhythmia, and 41 cardiomyopathy-associated genes	For novel nonsense, frameshift, and splice-site variants, the characteristics of the gene and the variant, and the participant's family history, were considered. Variants were designated class 4 (likely pathogenic) if there was a single case reported as pathogenic with supporting evidence (ie, segregation, absent in controls, functional studies),	Novel missense or in-frame insertion/deletion variants were assigned to class 3 (variant of uncertain significance). Missense and in-frame insertion/deletion variants reported a single time as pathogenic without supporting evidence or	We reasoned that no single allele with a frequency of >0.01 in ClinSeq or Single Nucleotide Polymorphism Database minor allele frequency of >0.015 (Single Nucleotide Polymorphism Database[7] build 132, minimum 120 chromosomes) could cause a disorder with a prevalence of 1/500 and designated these class 1.

					2 cases reported as pathogenic without additional evidence for or against pathogenicity, or ≥3 cases without sufficient race-matched control data to exclude a high population frequency. Class 5 was assigned when 2 cases with additional supporting evidence were presented or ≥3 cases were reported as pathogenic without evidence against pathogenicity and with sufficient race-matched control data to exclude a high population frequency. Two investigators analyzed variants and assigned a consensus pathogenicity score.	multiple times with evidence against pathogenicity and loss of function (ie, nonsense, frameshift, or splice-site) variants reported once with single evidence against pathogenicity or multiple times with multiple evidence against pathogenicity were also assigned to class 3.	
Nomura et al., 2016	D,S	7, 600	HCM		six independent filters were applied to facilitate detection of causal variants among the		Together with CADD score and HHE gene data, WES facilitated successful identification of the one causative variant, the MYL3 Arg94His. prediction of the pathogenicity of human variants with the CADD C-score is more accurate than that of any other ***in silico*** single annotation tool.

					enrolled HCM families. Variants were filtered by: (1) minor allele frequency (MAF) >1% in Asian population; (2) benign, as predicted by SnpEff; (3) genotype–phenotype unmatched under the assumption of complete penetrance without phenocopies; (4) registered in the SNP Database (dbSNP137); (5) combined annotation dependent depletion (CADD) score <10; and (6) low heart expression of the genes, less than the top quartile.Prediction of ***in silico*** pathogenicity for novel missense variants was performed using the CADD prediction software (version 1.0), which objectively integrates many diverse annotations into a		

					single measure (C-score) for each variant. A variant was predicted to be pathogenic if the scaled C-score calculated by the software was above 10, a score indicative of the variant being within the top 10% of deleteriousness substitutions. Candidate variants were evaluated if the gene associated with each variant was directly involved in the myocardium or worsening cardiac function by using the high heart expression (HHE) gene data		
Obeyesekere et al., 2012	D	26	LQTS	***KCNQ1; KCNH2; KCNE1, KCNE2; AKAP9***	Class I: deleterious or predicted deleterious; Class II: VUS – mutation may be disease-causing or benign, evidence is insufficient to determine deleteriousness; Class III: not expected to cause disease, typically common in a healthy control population.	VUS defined as variant typically absent from a healthy control population and may be disease-causing. VUS carriers present with substantially longer end-recovery QTc	End-recovery QTc, along with LQT mutation type and location, is a useful metric for interpretation of VUS associated with LQTS.

						compared to controls.	
Selga et al., 2015	D	55	BrS	***SCN5A; CACNA1C; CACNB2; GPD1L; SCN1B; SCN2B; SCN3B; SCN4B; KCNE3; RANGRF; HCN4; KCNJ8; KCND3; KCNE1L***	Rare (MAF<1%) variants previously described as associated with BrS, stop and frameshift variants considered potentially pathogenic variants. Genetic variations with MAF >1% considered benign.	Variation with a potential modifier effect on the phenotype but not clearly responsible for the disease.	The effects of common genetic variations as phenotypic modifiers are gradually becoming more and more important in the explanation of certain phenotypes of genetic diseases.
Steffensen et al., 2015	D	39	LQTS	***KCNQ1; KCNH2; SCN5A; KCNE1; KCNE2***	Based on 2008 ACMG classification recommendations	Based on 2008 ACMG classification recommendations	With greater appreciation of the extent of rare genetic variation seen in the general population, the approach is more conservative in calling pathogenic mutations which adds to the VUS rate
van Spaendonck-Zwarts et al., 2014	D	18 families	PPCM; DCM	48 cardio-myopathy related genes	Classified mutations as: pathogenic, not pathogenic, or as a variant of unknown clinical significance (VUS). Used a list of mutation-specific features based on ***in silico*** analysis. Co-segregation data and/or functional analysis were needed to classify a mutation as pathogenic.	Variants of unknown clinical significance (VUS) classified as: VUS1, unlikely to be pathogenic; VUS2, uncertain; VUS3, likely to be pathogenic	Our study has several advantages: one is the inclusion of some large families, where co-segregation analysis added value to the classification of mutations. Another was the large number of genes we tested, including the large ***TTN*** gene, for which mutation analyses on a large scale were impossible before NGS became available
van de Luijtgaarden et al., 2015	D, S	155	AAA; Inherited thoracic aortic aneurysm	***ACTA2; COL3A1; EFEMP2; FBN1; MYH11;***	Five classes: pathogenic, likely pathogenic, unknown significance (VUS),	A single previous description of a variant in a patient	Establishing a causal effect of variants involves finding a method of choice for functional testing of variants in aneurysm genes, which is complicated giving the likelihood of tissue-specific gene expression. Especially since nowadays abdominal aortic aneurysms are mostly restored by an endovascular procedure, no aortic aneurysm tissue from patients can be collected for functional testing.

				MYLK; SMAD3; TGFB2; TGFBR1; TGFBR2; one variant in ***MTHFR***	likely benign, and benign. The criteria for classification of variants included the allele frequency in the dbSNP/ESP (cutoff 0.01), predicted effects on splicing, the in silico prediction of effect on the protein and previously described links to disease. A variant only predicted by in silico prediction to be pathogenic would not automatically be classified as such because of lack of functional evidence	was not considered as sufficient evidence for causation and these variants were classified as variants of unknown significance. Intronic, silent or missense variants that affect splicing, in-frame deletions/insertions, missense variants for which more than 2 in silico protein predictions are damaging	
Wang et al., 2014	D	529	HCM	***MYH7; MYBPC3; TNNT2; TNNI3; MYL2; MYL3; TPM1; ACTC1***	Common polymorphisms and likely neutral variants excluded based on: MAF≥1%, detection in healthy controls, benign prediction by PolyPhen-HCM and PolyPhen-2, and no effect on splice site by Human Splicing Finder. Variant defined as novel if absent in HGMD.	Not addressed	It is possible some neutral rare variants were included in study as most of the novel variants were not confirmed by co-segregation or functional studies.

Walsh et al., 2014	D	2266	BrS; CPVT	***SCN5A; RYR2***	Paralogue (evolutionary related genes) annotation: A variant that is known to be pathogenic in one member of a protein family is used to annotate the equivalent amino acid of other members of the family for which no clinical genetic information exists	Variants classified as VUS or novel if they had not previously been reported in the literature and have not been confirmed in an independent study.	This approach was developed and experimentally validated by application to a large set of known variants in eight LQTS genes, and was found to have a positive predictive value (PPV) of 98.4% in these genes
Zhao et al., 2015	S	21	DCM	***MYBPC3, MYH6, MYH7, LMNA, TNNT2, TNNI3, MYPN, MYL3, TPM1, SCN5A, DES, ACTC1 and RBM20***	Putative pathogenic mutations were considered to be pathogenic based on the following criteria: i) the mutation has been reported to be associated with the disease phenotype in the reference or Human Gene Mutation Database; ii) the mutation has a minor allele frequency (MAF) of <1% in the NCBI dbSNP Build 137, the 1000 Genomes Project, and the National Heart, Lung, and Blood Institute Exome Sequencing Project	Control alleles (n=200) were taken from 100 unrelated healthy subjects with normal phenotypes (matched for gender, age and ethnicity) to exclude the possibility of rare polymorphisms of the novel mutations and variants of uncertain significance. there were 12 probands (patients	Online bioinformatics software (PolyPhen-2, SIFT and MutationTaster) was used to predict the functional effects of the altered proteins in the DCM patients, (seven novel mutations and three variants of uncertain significance). The mutations were predicted to localize to the functional region of the proteins

					databases; iii) the protein structure and function was significantly altered, and the amino acid was highly conserved across a number of species; iv) the mutation was analyzed and shown to be pathogenic using the PolyPhen-2, SIFT, or MutationTaster algorithms; or v) novel mutation or variants of uncertain significance had to be absent from unrelated and healthy controls which were matched for ethnicity	with DCM) harbouring one mutation (12/21, ~57.2%), including the 7 novel mutations, 3 variants of uncertain significance, and 2 previously reported mutations.	
Ziganshin et al., 2015	D	102	TAAD	21-gene panel	Variants classified according to likelihood that they were causative of the patient's phenotype. Probable Disease-Causing Mutation: Alterations in genes known to cause disease by loss of function; predicted to disturb encoded protein; well-established	VUS designation was used for rare missense alterations and in-frame deletions affecting highly conserved amino acids, which did not meet the criteria listed for disease-causing	At present, counseling patients with identified variants of unknown significance presents a challenge. Even though the identified variants are mutations within genes known to cause thoracic aortic disease, it is not yet known whether that particular mutation is disease causing.

					association with disease based on multiple independent studies, functional assays, or demonstration of abnormal messenger mRNA transcript processing.	mutation.	

VARIANT CLASSIFICATION

Presented here is a summary of evidence used in the 32 publications to assess pathogenicity of variants in genes associated with SCD-related disorders. Following the outline of a major workshop on genetic causality (MacArthur et al., 2014), we divide this into two sections – the validity of the disease-gene association and the validity of the variant-pathogenicity assertion.

Disease-gene association validity

The validity of the gene-disease association should be confirmed before assessing the pathogenicity of variants within that gene because not all gene-disease associations are valid (Andreasen et al., 2013; Kenna et al., 2013; Xue et al., 2012). The implication of a gene in a disease needs to be vetted on multiple criteria such as genetic evidence, frequency genetic burden test of some type (assessing the prevalence of mutations in affecteds and unaffecteds, functional assays, and other types of evidence. None of the 32 papers formally evaluated the validity of the gene-disease relationship. These publications assumed validity of the relationship of *RYR2* to CPVT – catecholaminergic polymorphic ventricular tachycardia; *SCN5A* to BrS – Brugada syndrome and LQTS – long QT syndrome; *MYH7* and *MYBPC3* to HCM – hypertrophic cardiomyopathy; *DCS2, DSG2, PKP2,* and *DSP* to ARVC – arrhythmogenic right ventricular cardiomyopathy; *FBN1* to Marfan syndrome; *TGFB2, TGFBR1,* and *TGFBR2* to Loeys-Dietz syndrome.

Evidence Used to Assess Variant Pathogenicity

Population frequency

Variants that cause moderately to highly penetrant Mendelian disorders are generally rare. Minor allele frequency (MAF) from genetic databases was used by all 32 studies to identify rare variants or exclude common variants. Commonly consulted public databases include: 1K Genomes Project, NHLBI ESP Exome Variant Server, HapMap, dbSNP, and more recently, the Exome Aggregation Consortium (ExAC). These databases typically include unselected individuals, but may include subclinical or asymptomatic individuals with potentially pathogenic variants but are still tremendously informative in determining variant frequencies. A few databases are enriched for patients with cardiovascular disease, which can complicate variant frequency assessments. The prevalence of genetic variants previously associated with SCD-related disorders in some of these databases suggests that these variants either have incomplete penetrance or are most likely not monogenic causes of associated diseases (Yang, Jabbari, Cheng, et al., 2014; Jabbari, Jabbari, Nielsen, et al., 2013). Another explanation is that the database includes individuals ascertained for these diseases (Richards et al., 2013). Allele frequencies from in-house or private exome data were used to filter variants in some studies (Bagnall et al., 2014; Ng et al., 2013).

Genetic Segregation

Nine (9) articles evaluated cosegregation information in determining variant pathogenicity (Das et al., 2014; Campuzano et al., 2014; Garcia-Pavia, 2011; Hershberger et al., 2009; Hofman et al., 2013; Obeyesekere et al., 2012; Selga et al., 2015; van Spaendonck-Zwarts et al., 2014; Ziganshin et al., 2015). In Das et al, a panel of four experts performed a blinded independent review of the pathogenicity evidence and reclassification of variants identified in HCM

probands using current and updated cosegregation data (Das et al., 2014). Cascade screening of relatives was used by 6 studies to assess segregation of variants (Hershberger et al., 2009; Hofman et al., 2013; Obeyesekere et al., 2012; Selga et al., 2015; van Spaendonck-Zwarts et al., 2014; Ziganshin et al., 2015). As with most family studies in age-dependent disorders, incomplete penetrance in some families with young members with potentially pathogenic variants could not be established (Selga et al., 2015).

Prior to the advent of high-throughput sequencing, rare monogenic (Mendelian) variants were identified using the positional cloning approach. As cosegregation is direct evidence of linkage of a locus to a phenotype and indirect evidence of pathogenicity of the variant itself, the American College of Medical Genetics and Genomics (ACMG) score cosegregation with disease in multiple affected family members as supporting evidence of pathogenicity. Conversely, lack of segregation of an identified variant with disease is strong evidence against pathogenicity (Richards et al., 2015).

Computational predictive algorithms

Twenty-four (24) of the 32 studies utilized one or multiple predictive algorithms to predict the functional effects of genetic variants. These computational algorithms are broadly speaking, based on changes to: conservation sequences, domain structure and function, splice sites, molecular mechanism disruption, and biochemical properties of the amino acids affected. Predictions from these algorithms are typically not included in clinical reports communicated to ordering clinicians as they are currently considered to lack the maturity (accuracy) to be clinically applicable. Commonly used computational tools include: Align GVGD, CONDEL,

PolyPhen-2, Mutation Taster, MutPred, PMUT, SIFT, Grantham, CADD, NNSPLICE, the maximum entropy model (MaxEntScan), and GeneSplicer.

The ACMG recommends that predictions combined from different algorithms be considered a single piece of evidence in variant interpretation (Richards et al., 2015). Akilzhanova and colleagues employed 5 prediction tools in their classification of variants found in known *RYR2* gene mutation hotspots in 35 patients with CPVT phenotypes. Missense variants were classified as possibly damaging when at least three of the five prediction algorithms scored variant as possibly damaging. A variant was called a VUS when three or less prediction algorithms (but at least one positive entry) scored variant as possibly damaging (Akilzhanova et al., 2014). To address the issue of rare missense background noise rate (3-8%) in LQTS genetic testing, Giudicessi and colleagues employed the predictive power of four *in silico* tools (Conservation, Grantham, SIFT, and PolyPhen) to create a composite score of the number of predictive tools in agreement on pathogenicity classification (0-4). The synergistic approach, with agreement on variant pathogenic status in 3 of the 4 algorithms, improved the estimated predictive values (EPV) for interpretation of tested results from 56% to 91% (Giudicessi et al., 2012). Calculating the agreement of multiple prediction algorithms to determine pathogenicity has also been used in other studies (Yang, Jabbari, Cheng, et al., 2014; Jabbari, Jabbari, Nielsen, et al., 2013).

The limitations of using predictive models to classify variant is dependent in part on which models are combined to generate a composite score. In the study by Giudicessi, Conservation and Grantham matrix predicted that only 52% and 41% of case-derived missense variants are potentially pathogenic, while SIFT and PolyPhen predicted that 28% and 46% of

control-derived missense variants are potentially pathogenic. Deciding on which algorithms to combine could depend on which mistake (false-positive or negative) one is more comfortable making.

To improve sensitivity and specificity, disease-specific and gene-specific predictive tools are designed and validated using variants classified with established clinical criteria by authoritative repositories of gene variants with clear association with disease phenotypes (Crockett et al., 2011; Jordan et al., 2011). Jordan and colleagues used 74 manually and systematically classified missense variants to develop and validate a disease-specific computational algorithm to predict pathogenicity in six HCM-related genes (Jordan et al., 2011). Coverage of the prediction tool was estimated at 57%. The designed prediction tool does not accurately predict the effect of splice site variants, and it remains an open question how generalizable an algorithm trained on a small data set will be in predicting variants outside of the six HCM-associated genes.

With an initial focus on genes associated with inherited arrhythmias, a method called paralogue annotation identifies functional residues intolerant of variation in families of evolutionary related proteins. Variants known to be pathogenic in one member of a protein family is used to annotate the equivalent amino acid (paralogue) of the other members of the family for which no genetic information or interpretation is available (Chung et al., 2009; Walsh et al., 2014). This link between genetic variation and clinical phenotype in paralogues of disease genes is currently not widely used in variant annotation. With a positive predictive value (PPV) of 98.4%, this approach provides a web-based tool to identify paralogue annotations for novel variant classification and interpretation in genes associated with monogenic diseases. Variants

previously classified as VUS's in SCD-related disorders could potentially be reclassified with this information as additional pathogenicity evidence. Accuracy of paralogue annotation is greatly dependent on the quality of alignment of the amino acids regarded as functionally equivalent and the quality of evidence reported on the paralogue variant.

Predictive algorithms, although less used as such, predict variants as benign. This is a supporting evidence for classifying variant as benign in the ACMG guidelines. Wang and colleagues excluded novel missense variants predicted to be benign from their genotype-phenotype correlation analysis based on predictions by PolyPhen-HCM/2, SIFT, and Human Splicing (Wang et al., 2014).

Although the assumptions and sources of information used to design most predictive tools are similar in many respect, some tools may not be reliable for particular datasets. Gene-specific predictive models offer significantly enhanced performance by highlighting important mutation characteristics specific to the gene used to design and train the algorithm. It is recommended that multiple algorithms be used to check for consensus or conflict in predicting variant deleteriousness or damaging effects. Predictive algorithms can be useful in guiding variant classification but appropriate caution is necessary when using their predictions as supporting evidence (Tchernitchko et al., 2004; Richards et al., 2015).

Mutation type and location

In an effort to address the interpretability of missense variants, which make up the bulk of genetic background noise, 3 studies (Kapa et al, 2009; Giudicessi et al, 2012; and Selga et al) assessed the locations (gene regions) in which missense variants in the three main LQTS-

associated genes (*KCNQ1, KCNH2, SCN5A*) were found in LQTS cases and otherwise healthy controls. In the absence of functional and expression studies to verify the effects of missense variants, calculating the EPV (probability that a variant found in a case is disease-causing) based on the gene region harboring the variant provides an estimate of pathogenicity likelihood. The use of channel structure-function domains to determine the pathogenic probability of novel missense variants requires a fairly large control population to establish accurate and confident values in regions and domains not addressed by these studies. The rare variants found in controls, as noted by the authors, are not all benign as some of the mutations may in fact not be genetic noise but incompletely penetrant variants. A large cohort of truly healthy controls would have to be described to effectively use this approach for missense variant classification. It is also worth noting that in a mutational analysis of the cardiac sodium channel by Ackerman and colleagues to determine the prevalence of *SCN5A* nonsynonymous polymorphisms in four distinct healthy ethnic controls, rare genetic missense variants considered benign localized to interdomain linker regions of the $Na_v1.5$ cardiac sodium channel. Additionally, these domain linkers host 16% and 26% of reported BrS-associated and LQT3-associated missense variants respectively (Ackerman et al., 2004). Also, the potentially pathogenic variants identified by Selga and colleagues were mainly located in the extracellular loops and interdomain linker regions (Selga et al., 2015). In the absence of additional functional evidence, about 5% of apparently healthy individuals have a rare missense variant in the cardiac sodium channel (Kapplinger et al., 2015). This overlap in location of polymorphisms and potentially pathogenic variants presents a challenge to using variant location to classify variants. According to the

ACMG guidelines, a mutational hotspot or well-established functional domain must lack benign variants (Richards et al., 2015).

Functional studies

Studies to determine the functional and electrophysiological differences between pathogenic and benign variants can provide additional information needed for further variant assessment. Using current methodologies, it is impractical to perform functional studies on every ion channel or sarcomeric gene variant(Kapa et al, 2009). Five (5) studies employed functional assays in their variant classification approaches (Steffensen et al., 2015; Gregers Winkel et al., 2015; Groeneweg et al., 2014; Obeyesekere et al., 2012; van Spaendonck-Zwarts et al., 2015). As with most *in vitro* analyses, conclusions were limited in their validity to *in vivo* conditions. For example, pre-mRNA processing is tissue specific; therefore the evaluation of spliceogenic potential of variants in peripheral blood leukocytes may not fully translate to cardiac tissue In a study to identify potentially causal mutations in families with both peripartum cardiomyopathy and dilated cardiomyopathy, heart tissue from a patient with a *TTN* variant was functionally analyzed by measuring passive force in single cardiomyocytes at sarcomere lengths of 1.8 to 2.2µm (van Spaendonck-Zwarts et al., 2015). The procurement of heart tissue for functional analyses of a variant, especially a VUS, will doubtlessly limit widespread application of passive force measurement. Analytical performances of the assay and specimen integrity are important factors to consider in evaluating the robustness of the data generated by a functional assay (Richards et al., 2015).

DISCUSSION

The ACMG standardized variant classification process is, though not fully integrated, slowly permeating genetic research and clinical analyses. Assessment of the association evidence between a gene and disease should be the initial step in genetic variant classification. Two articles in this review revisited the disease-gene association evidence in genes previously linked to SCD-related disorders. This step should be revisited periodically to maintain the scientific integrity of the reported evidence as more population and disease-specific databases come online. Reviewing the literature on gene-phenotype association allows for better characterizations of disease phenotype. Mode of inheritance, region of a gene where a variant is located, and different types of variants in the same gene may be associated with different phenotypes (Duzkale et al., 2013).

A portion of SCD-related disorders will be explained by variants in yet to be identified genes, but the proportion currently explained by previously associated genes should be the starting point for investigating disease causality. For example, loss of function mutations in *SCN5A*-encoded cardiac sodium channel have been documented to be responsible for about 30% of Brugada syndrome (BrS) cases (Alings & Wilde, 1999), and gain-of-function mutations in the cardiac ryanodine receptor encoded by *RYR2* are responsible for over 50% of CPVT cases (Priori, Napolitano, Memmi, et al., 2002). Genes with unreliable disease correlations undermine the best efforts to standardize variant classification, and impede the possibilities of wider clinical utility of genetic testing. It is worth noting that computational algorithms are trained and tested using gene-disease attributes generated on the basis of prior gene-disease association.

Evidence used to assess variant pathogenicity includes population frequency, genetic cosegregation, predictive algorithms, mutation type and location, and functional studies. ACMG recommends that clinical laboratories work with experts in statistical and population genetics to analyze the significance of variant-phenotype association, and expert judgment can be used to evaluate the full body of evidence implicating a variant to a phenotype (Richards et al., 2015).

All the reviewed articles consulted public databases to establish the population frequency of their variant of interest. There is ample evidence of population databases containing pathogenic variants from subclinical and asymptomatic individuals that inadvertently skew the reported allele frequencies of rare variants. These databases can be the initial sources of information on variant frequency but should be approached and consulted with caution (Richards et al., 2015).

Cosegregation analysis added significant value to the variant classification process of 9 studies in this review. Variant cosegregation with disease in multiple affected family members is supporting evidence of pathogenicity, and relatives that test negative for the variant are spared the burden of on-going screening. (Garcia-Pavia et al., 2011; Das et al., 2014; Selga et al., 2015). A limitation to cosegregation information is the loss of affected family members with SCD-related diseases at a young age (Pilmer et al., 2014), leading to insufficiently powered segregation analyses in families.

Predictive algorithms provide reasonably accurate information on genetic variants based on predicted deleteriousness or impact on splice site sequences. Twenty-four (24) out of the 32 studies included in this review used one or multiple predictive algorithms in their variant

annotation Some have argued that most of these predictive algorithms suffer from poor sensitivity and specificity, as they are not developed with clinical application in mind and are not carefully validated against well-curated, manually classified pathogenic and benign variants (Crockett et al., 2011; Jordan et al., 2011). Disease-specific and gene-specific predictors have better accuracy by focusing on a specific disease mechanism and common variations found in a gene but the methods underlying their development is similar to other widely used predictors. This presents a choice between accuracy and generalizability. The study by Jordan et al on the development and training of a specialized predictor algorithm for HCM using a dataset of 74 variants reported a median accuracy of 92%, compared with 74% and 70% for SIFT and PolyPhen-2 respectively (Jordan et al., 2011). The utility of prediction algorithms reside in their ability to annotate variants that other tests and manual processes are unable to determine. It is difficult to establish how high a confidence to allot to a specialized predictor trained on a low number of missense variants, and how low a confidence to place in a general-purpose predictor trained without prior knowledge of the disease of interest. With predictive algorithms relying on similar data to support their predictions, care must be taken to place measured and appropriate significance on computational evidence (Richards et al., 2015).

The location of a variant within a gene's structure can provide some information on its pathogenicity. The estimated predictive value (EPV) for certain types of variants (insertion, deletion, nonsense) range from 95% to almost 100% regardless of their gene location. This is not the case for missense variants. The EPV for missense variants is dependent on the specific structure-function domain to which it localizes (Guidicessi et al., 2012). As an example, the

overall EPV of missense variants that alter conserved sequences in the *KCNQ1 and KCNH2* genes was reported at 97% by Giudicessi et al, and between 71% and 96% for missense variants that localize to the transmembrane region of the same genes by Kapa et al. Calculating the EPV for missense variants in a specific gene region is not a diagnosis of disease, it provides an upper boundary on the certainty of variant pathogenicity that can inform classification in the absence of other conclusive or supporting evidence (Kapa et al., 2009).

The knowledge and understanding of the location of missense variants in gene-encoded regions does not eliminate the issue of false positive variant classification as potentially pathogenic variants described to cause dysfunction to cardiac sodium channels in BrS patients have been reported in regions of voltage-gated channels proposed to be benign (Selga et al., 2015). ACMG recommends that information on missense variants identified in protein domains determined to be critical to protein function, with no benign variants reported in same domain, can be considered moderate evidence (PM1) of pathogenicity (Richards et al., 2015).

Few causative variants or alterations are functionally investigated in SCD-related disorders with either *in vivo* studies in animal models or *in vitro* studies to generate pathogenicity evidence. When functional evidence is available, it is critical to examine whether the assay design and conclusions apply to the mechanism(s) of the disease being investigated (Duzkale et al., 2013). Testing laboratories consider functional evidence in their variant classification decision tree. What weight is given to the assays used to generate functional evidence vary by laboratory. *In vitro* evidence that a variant disrupts normal function is arguable good classification evidence, but the challenge is refuting the possibility that some other variant is responsible or contributing to the dysfunction. How closely a functional assay

reflects the biological environment is a key considering in assessing the validity of a functional assay (Richards et al., 2015). Patient tissue at the system level may not be readily accessible in some conditions such as aortic aneurysms and dissections (van de Luijtgaarden et al., 2015), and in relation to most SCD-related disorders heart tissue may be the preferred tissue of choice (van Spaendonck-Zwarts et al, 2014). With extensive reproducibility and validation, functional characterization can be a significant source of evidence in the variant classification process. Well-established functional studies that replicate the damaging impact of a variant on protein function can be powerful and strong evidence (PS3) in support of pathogenicity (Richard et al., 2015).

This review highlights the variety of approaches by laboratories in their variant assessment process. The ACMG guidelines provide a framework for genetic testing laboratories with the ultimate goal of standardizing the variant classification process. Although more needs to be done to establish a uniform system across laboratories, an encouraging sign is that the five-tier terminology system was used by most of the studies in this review. Future variant classification reviews may report better uptake of the recommended standards and guidelines.

CONCLUSIONS

Clinical application of genetic information remains a key tool to reducing and ultimately preventing the incidence of SCD in the general population. Re-assessment of information at the gene and variant levels is critical to strengthening the validity of the implicating evidence. Population, segregation, predictive, functional, and mutation location data are weighted differently by testing laboratories, leading to inconsistencies in variant classification in SCD-

related genes and cardiac genetics more broadly. The ACMG recommendations provide an evidence framework that could standardize variant classification. Generating supporting information using functional studies, mutation location, and predictive algorithms for variants implicated in SCD-related disorders remain areas that can benefit from further investigation, especially for variants with limited population and segregation level genetic evidence.

References

Ackerman, M.J., Priori, S.G., Willems, S., Berul, C., Brugada, R., Calkins, H., et al. (2011). HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Eurospace,* 13(8), 1077-109.

Ackerman, M.J., Splawski, I., Makielski, J.C., Tester, D.J., Will, M.L., Timothy, K.W., et al. (2004). Spectrum and prevalence of cardiac sodium channel variants among black, white, Asian, and Hispanic individuals: implications for arrhythmogenic susceptibility and Brugada/long QT syndrome genetic testing. *Heart Rhythm,* 1(5), 600-607.

Adabag, A.S., Luepker, R.V., Roger, V.L., & Gersh, B.J. (2010). Sudden cardiac death: epidemiology and risk factors. *Nat Rev Cardiol,* 7, 216-225.

Adzhubei, I.A., Jordan, D.M., & Sunyaev ,S.R. (2013). Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc *Hum Genet*, Chapter 7, Unit 7.20.

Akilzhanova, A., Guelly, C., Nuralinov, O., Nurkina, Z., Nazhat, D., Smagulov, S., et al. (2014). RYR2 sequencing reveals novel missense mutations in a Kazakhidiopathic ventricular tachycardia study cohort. *PLoS One,* 9 (6), e101059.

Alings, M., & Wilde, A. (1999). Brugada syndrome: clinical data and suggested pathophysiological mechanism. *Circulation*, 99, 666-673.

Andreasen, C., Nielsen, J.B., Refsgaard, L., Holst, A.G., Christensen, A.H., Andreasen, L., et al. (2013). New population-based exome data are questioning the pathogenicity of previously cardiomyopathy-associated genetic variants. *Eur J Hum Genet*, 21(9), 918-928.

Bagnall, R.D., Das, K.J., Duflou, J., & Semsarian, C. (2014). Exome analysis-based molecular autopsy in cases of sudden unexplained death in the young. *Heart Rhythm,* 11(4), 655-662.

Biesecker, L.G., Mullikin, J.C., Facio, F.M., Turner, C., Cherukuri, P.F., Blakesley, R.W., et al. (2009). The ClinSeq Project: Piloting large-scale genome sequencing for research in genomic medicine. Genome Res, 19, 1665–1674.

Campuzano, O., Allegue, C., Fernandez, A., Iglesias, A., & Brugada, R. (2015). Determining the pathogenicity of genetic variants associated with cardiac channelopathies. *Sci Rep,* 5, 7953.

Campuzano, O., Sarquella-Brugada, G., Mademont-Soler, I., Allegue, C., Cesar, S., Ferrer-Costa, C., et al. (2014). Identification of genetic alterations, as causative genetic defects in long QT syndrome, using next generation sequencing technology. *PLoS One,* 9 (12), e114894.

Crockett, D.K., Lyon, E., Williams, M.S., Narus, S.P., Facelli, J.C., & Mitchell, J.A. (2011). Utility of gene-specific algorithms for predicting pathogenicity of uncertain gene variants. *J Am Med Inform Assoc*, 19(2), 207-211.

D'Argenio, V., Frisso, G., Precone, V., Boccia, A., Fienga, A., Pacileo, G., et al. (2014). DNA sequence capture and next-generation sequencing for the molecular diagnosis of genetic cardiomyopathies. *J Mol Diagn,* 16(1), 32-44.

Das, K.J., Ingles, J., Bagnall, R.D., & Semsarian, C. (2014). Determining the pathogenicity of genetic variants in hypertrophic cardiomyopathy: importance of periodic reassessment. *Genet Med,* 16(4), 286-293.

de Vreede-Swagemakers, J.J., Gorgels, A.P., Dubois-Arbouw, W.I., van Ree, J.W., Daemen, M.J., Houben, L.G., et al. (1997). Out-of-hospital cardiac arrest in the 1990's: a population-based

study in the Maastricht area on incidence, characteristics, and survival. *J Am Coll Cardiol,* 30, 1500-1505.

Di Resta, C., Pietrelli, A., Sala, S., Della Bella, P., De Bellis, G., Ferrari, M., et al. (2015). High-throughput genetic characterization of a cohort of Brugada syndrome patients. *Hum Mol Genet,* 24(20), 5828-5835.

Duzkale, H., Shen, J., McLaughlin, H., Alfares, A., Kelly, M.A., Pugh, T.J., et al. (2013). A systematic approach to assessing the clinical significance of genetic variants. *Clin Genet* 84(5), 453-463.

Eckart, R.E., Shry, E.A., Burke, A.P., McNear, J.A., Appel, D.A., Castillo-Rojas, L.M., et al. (2011). Sudden death in young adults: an autopsy-based series of a population undergoing active surveillance. *J Am Coll Cardiol,* 58, 1254-1261.

EMBL-EBI-1000 Genomes website. Available: www.browser.1000genomes.org.

Exome Aggregation Consortium (ExAC) website. Available: http://exac.broadinstitute.org.

Farrugia, A., Keyser, C., Hollard, C., Raul, J.S., Muller, J., & Ludes, B. (2015). Targeted next generation sequencing application in cardiac channelopathies: analysis of a cohort of autopsy-negative sudden unexplained deaths. *Forensic Sci Int,* 254, 5-11.

Garcia-Pavia, P., Syrris, P., Salas, C., Evans, A., Mirelis, J.G., Cobo-Marcos, M., et al. (2011). Desmosomal protein gene mutations in patients with idiopathic dilated cardiomyopathy undergoing cardiac transplantation: a clinicopathological study. *Heart,* 97(21), 1744-1752.

Giudicessi, J.R., Kapplinger, J.D., Tester, D.J., Alders, M., Salisbury, B.A., Wilde, A.A., et al. (2012). Phylogenetic and physicochemical analyses enhance the classification of rare

nonsynonymous single nucleotide variants in type 1 and 2 long-QT syndrome. *Circ Cardiovasc Genet,* 5(5), 519-528.

Gonzalez-Torrecilla, E., Arenal, A., Atienza, F., Datino, T., Bravo, L., Ruiz, P., et al. (2015). Current indications for implantable cardioverter defibrillators in non-ischemic cardiomyopathies and channelopathies. *Rev Rec Clin Trials,* 10, 111-127.

Gradman, A., Deedwania, P., Cody, R., Massie, B., Packer, M., Pitt, B., et al. (1989). Predictors of total mortality and sudden cardiac death in mild to moderate heart failure. Captopril Digoxin Study Group. *J Am Coll Cardiol,* 14, 564-570.

Grimm, W., Christ, M., Bach, J., Muller, H.H., & Maisch, B. (2003). Noninvasive arrhythmia risk stratification in idiopathic dilated cardiomyopathy results of the Marburg Cardiomyopathy Study. *Circulation,* 108, 2883-2891.

Groeneweg, J.A., Ummels, A., Mulder, M., Bikker, H., van der Smagt, J.J., van Mil, A.M., et al. (2014). Functional assessment of potential splice site variants in arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Heart Rhythm,* 11(11), 2010-2017.

Helbig, I., Hodge, S.E., & Ottman, R. (2013). Familial cosegregation of rare genetic variants with disease in complex disorders. *Eur J Hum Genet*, 21, 444-450.

Hertz, C.L., Christiansen, S.L., Ferrero-Miliani, L., Dahl, M., Weeke, P.E., LuCamp., et al. (2015). Next-generation sequencing of 100 candidate genes in young victims of suspected sudden cardiac death with structural abnormalities of the heart. Int J Legal Med, 130(1), 91-102.

Hoffmann, B.A., Rybczynski, M., Rostock, T., Servatius, H., Drewitz, I., Steven, D., et al. (2013). Prospective risk stratification of sudden cardiac death in Marfan's sundrome. *Int J Cardiol,* 167(6), 2539-2545.

Hofman, N., Tan, H.L., Alders, M., Kolder, I., de Haij, S., Mannens, M.M., et al. (2013). Yield of molecular and clinical testing for arrhythmia syndromes: report of 15 years' experience. *Circulation,* 128 (14), 1513-1521.

Jabbari, J., Jabbari, R., Nielsen, M.W., Holst, A.G., Nielsen, J.B., HaunsØ, S., et al. (2013). New exome data question the pathogenicity of genetic variants previously associated with catecholaminergic polymorphic ventricular tachycardia. *Circ Cardivasc Genet*, 6(5), 481-489.

Johnston, J.J., Rubinstein, W.S., Facio, F.M., Ng, D., Singh, L.N., Teer, J.K., et al. (2012). Secondary Variants in Individuals Undergoing Exome Sequencing: Screening of 572 Individuals Identifies High-Penetrance Mutations in Cancer-Susceptibility Genes. *Am J Hum Genet*, 91, 97–108.

Jordan, D.M., Kiezun, A., Baxter, S.M., Agarwala, V., Green, R.C., Murray, M.F., et al. (2011). Development and validation of a computational method for assessment of missense variants in hypertrophic cardiomyopathy. *Am J Hum Genet,* 88 (2), 183-192.

Kapplinger, J.D., Giudicessi, J.R., Ye, D., Tester, D.J., Callis, T.E., Valdivia, C.R., et al. (2015). Enhanced classification of Brugada syndrome-associated and Long-QT syndrome-associated genetic variants in the SCN5A-encoded Nav1.5 cardiac sodium channel. *Circ. Cardiovasc Genet* 8(4), 582-595.

Kapa, S., Tester, D.J., Salisbury, B.A., Harris-Kerr, C., Pungliya, M.S., Alders, M., et al. (2009). Genetic testing for long-QT syndrome: distinguishing pathogenic mutations from benign variants. *Circulation,* 120(18), 1752-1760.

Kapplinger, J.D., Tester, D.J., Salisbury, B.A., Harris-Kerr, C., Pollevick, G.D., et al. (2009). Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the familion long qt syndrome genetic test. *Heart Rhythm*, 6, 1297-1303.

Kenna, K.P., McLaughlin, R.L., Hardiman, O., & Bradley, D.G. (2013). Using reference databases of genetic variation to evaluate the potential pathogenicity of candidate disease variants. *Hum Mutat*, 34, 836–841.

Krawczak, M., Ball, E.V., Fenton, I., Stenson, P.D., Abeysinghe, S., Thomas, N., et al. (2000). Human gene mutation database - a biomedical information and research resource. *Hum Mutat*, 15, 45-51.

Lopes, L.R., Syrris, P., Guttmann, O.P., O'Mahony, C., Tang, H.C., Dalageorgou, C., et al. (2015). Novel genotype-phenotype associations demonstrated by high-throughput sequencing in patients with hypertrophic cardiomyopathy. *Heart,* 101 (4), 294-301.

MacArthur, D.G., Manolio, T.A., Dimmock, D.P., Rehm, H.L., Shendure, J., Abecasis, G.R., et al. (2014). Guidelines for investigating causality of sequence variants in human disease. *Nature,* 508(7497), 469-476.

Margulies, E.H., Green, E.D., Collins, F.S., Mullikin, J.C., Biesecker, L.G., Teer, J.K., et al. (2010). Systematic comparison of three genomic enrichment methods for massively parallel DNA sequencing. *Genome Res*, 20, 1420–1431.

Maron, B.J., Maron, M.S., & Semsarian, C. (2012). Genetics of hypertrophic cardiomyopathy after 20 years: clinical perspectives. *J Am Coll Cardiol*, 60, 705–715.

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., et al. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*, 20, 1297–1303.

NCBI-International HapMap Project website. Available: www.hapmap.ncbi.nlm.nih.gov/.

dbSNP database. Available: www.ncbi.nlm.nih.gov/SNP/.

Ng, D., Johnston, J.J., Teer, J.K., Singh, L.N., Peller, L.C., Wynter, J.S., et al. (2013). Interpreting secondary cardiac disease variants in an exome cohort. *Circ Cardiovasc Genet,* 6(4), 337-346.

NHLBI Exome Sequencing Project (ESP) Exome Variant Server website. Available: www.evs.gs.washington.edu/EVS/.

Nomura, A., Tada, H., Teramoto, R., Konno, T., Hodatsu, A., Won, H.H., et al. (2016). Whole Exome sequencing combined with integrated variant annotation prediction identifies a causative myosin essential light chain variant in hypertrophic cardiomyopathy. J Cardiol, 67(2), 133-139.

Obeyesekere, M.N., Sy, R.W., Klein, G.J., Gula, L.J., Modi, S., Conacher, S., et al. (2012). End-recovery QTc: a useful metric for assessing genetic variants of unknown significance in long-QT syndrome. *J Cardiovasc Electrophysiol,* 23(6), 637-642.

Pezawas, T., Diedrich, A., Winker, R., Robertson, D., Richter, B., Wang, L., et al. (2014). Multiple autonomic and repolarization investigation of sudden cardiac death in dilated cardiomyopathy and controls. *Circ Arrhythm Electrophysiol,* 7, 1101-1108.

Pilmer, C.M., Kirsh, J.A., Hildebrandt, D., Krahn, A.D., & Gow, R.M. (2014). Sudden cardiac death in children and adolescents between 1 and 19 years of age. *Heart Rhythm*, 11, 239-245.

Plon, S.E., Eccles, D.M., Easton, D., Foulkes, W.D., Genuardi, M., Greenblatt, M.S., et al. (2008). Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum Mutat*, 29, 1282-1291.

Priori, S.G., Napolitano, C., Memmi, M., Colombi, B., Drago, F., Gasparini, M., et al. (2002). Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. *Circulation*, 106, 69-74.

Priori, S.G., Schwartz, P.J., Napolitano, C., Bloise, R., Ronchetti, E., Grillo, M., et al. (2003). Risk stratification in the long-QT syndrome. *N Engl J Med,* 348*,* 1866–*1874.*

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., et al. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*, 17(5), 405-424.

Selga, E., Campuzano, O., Pinsach-Abuin, M.L., Perez-Serra, A., Mademont-Soler, I., Riuro, H., et al. (2015). Comprehensive genetic characterization of a Spanish Brugada syndrome cohort. *PLoS One,* 10 (7), e0132888.

Sherry, S.T., Ward, M.H., Kholodov, M., Baker, J., Phan, L., Smigielski, E.M., et al. (2001). dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res*, 29, 308-311.

Steffensen, A.B., Refaat, M.M., David, J.P., Mujezinovic, A., Calloe, K., Wojciak, J., et al. (2015). High incidence of functional ion-channel abnormalities in a consecutive Long QT cohort with novel missense genetic variants of unknown significance. *Sci Rep,* 5, 10009.

Tchernitchko, D., Goossens, M., & Wajcman, H. (2004). In silico prediction of deleterious effect of a mutation: proceed with caution in clinical genetics. *Clin Chem*, 50, 1974-1978.

van de Luijtgaarden, K.M., Heijsman, D., Maugeri, A., Weiss, M.M., Verhagen, H.J., IJpma, A., et al. (2015). First genetic analysis of aneurysm genes in familial and sporadic abdominal aortic aneurysm. *Hum Genet,* 134(8), 881-893.

van Spaendonck-Zwarts, K.Y., Posafalvi, A., van den Berg, M.P., Hilfiker-Kleiner, D., Bollen, I.A., Sliwa, K., et al. (2014). Titin gene mutations are common in families with both peripartum cardiomyopathy and dilated cardiomyopathy. *Eur Heart,* 35(32), 2165-2173.

Walsh, R., Peters, N.S., Cook, S.A., & Ware, J.S. (2014). Paralogue annotation identifies novel pathogenic variants in patients with Brugada syndrome and catecholaminergic polymorphic ventricular tachycardia. *J Med Genet,* 51(1), 35-44.

Wang, J., Wang, Y., Zou, Y., Sun, K., Wang, Z., Ding, H., et al. (2014). Malignant effects of multiple rare variants in sarcomere genes on the prognosis of patients with hypertrophic cardiomyopathy. *Eur J Heart Fail,* 16 (9), 950-957.

Winkel, B.G., Yuan, L., Olesen, M.S., Sadjadieh, G., Wang, Y., Risgaard, B., et al. (2015). The role of the sodium current complex in a nonreferred nationwide cohort of sudden infant death syndrome. *Heart Rhythm,* 12(6), 1241-1249.

Wooderchak-Donahue, W., VanSant-Webb, C., Tvrdik, T., Plant, P., Lewis, T., Stocks, J., et al. (2015). Clinical utility of next generation sequencing panel assay for Marfan and Marfan-like syndromes featuring aortopathy. *Am J Med Genet Part A*, 167A, 1747-1757.

Xue, Y., Chen, Y., Ayub, Q,, Huang, N., Ball, E.V., Mort, M., et al. (2012). Deleterious- and disease-allele prevalence in healthy individuals: insights from current predictions, mutation databases, and population-scale resequencing. *Am J Hum Genet*, 91, 1022–1032.

Yang, R.Q., Jabbari, J., Cheng, X.S., Jabbari, R., Nielsen, J.B., Risgaard, B., et al. (2014). New population-based exome data question the pathogenicity of some genetic variants previously associated with Marfan syndrome. *BMC Genetics*, 15, 74. doi: 10.1186/1471-2156-15-74.

Zhao, Y., Feng, Y., Zhang, Y.M., Ding, X.X., Song, Y.Z., Zhang, A.M., et al. (2015). Targeted next-generation sequencing of candidate genes reveals novel mutations in patients with dilated cardiomyopathy. *Int J Mol Med,* 36(6), 1479-1486.

Ziganshin, B.A., Bailey, A.E., Coons, C., Dykas, D., Charilaou, P., Tanriverdi, L.H., et al. (2015). Routine genetic testing for thoracic aortic aneurysm and dissection in a clinical setting. *Ann Thorac Surg,* pii: S0003-4975(15)00735-3.

CHAPTER THREE: METHODOLOGY

The purpose of this study was to describe the impact of returning genetic variants of uncertain significance result on recipients' health behavior intentions. The purpose was accomplished through a predictive correlational study that examined the relationships among perceived risk, perceived severity, perceived information value, self-efficacy, and health behavior intentions. The modifying effects of resilience, optimism, and tolerance of uncertainty on health behavior intentions were also examined. The methodology used to guide this study is described below.

Methods

Research Design

A predictive correlational design was used for this study. Participants with cardiomyopathy-associated genetic variants of uncertain significance (VUS) results were consented to receive their results. Results were disclosed to participants to assess their post-disclosure levels of perceived risk and perceived severity, perceived value of information, and self-efficacy. These factors were used to predict participants' intentions to pursue health-related behaviors. The influence of personality traits such as resilience, optimism, and tolerance of uncertainty on health behavior intentions was also examined.

Study Participants

Participants were recruited from a longitudinal study of genomic sequencing at the NIH (ClinSeq® project). The ClinSeq® project aims applies large-scale medical sequencing in a clinical context to address the genetic basis of health, disease, and drug response. The selection criteria for ClinSeq® participants and study design are published (Biesecker et al., 2009). Over 1000

Participants between 45 and 65 years old have been consented for initial phenotyping, exome and genome sequencing, and return of results. Enrolled participants were not preselected for family or personal history of arrhythmia, cardiomyopathy, or sudden cardiac death. Participants with a range of coronary artery disease risk were enrolled and grouped into 4 bins based on their 10-year Framingham risk calculation (<5%, 5%-10%, >10%, known coronary artery disease). Participants were told they would have the opportunity to learn the results from their exome and genome sequencing. Participants completed a baseline survey of risk perceptions and intentions to learn their sequencing results for various disease risks (preventable, non-preventable, carrier status) and how this information may change their health-related behaviors. Informed consent discussions with a genetic counselor described exome and genome sequencing, types of results to expect from genetic testing, choice to receive individual testing results as they become available, limitations in interpreting data, and the idea of uncertainty associated with genetic testing (Facio et al., 2013). This ancillary study was reviewed and approved by the NIH's National Human Genome Research Institute and the Johns Hopkins University Institutional Review Boards.

Cardiomyopathy Gene List

Variants of uncertain significance in a list of 41 cardiomyopathy-associated genes inherited in an autosomal pattern described by Ng et al. (2013) were used for this study. Briefly, nonsense, frameshift, splice-site, and nonsynonymous variants in the 41 identified genes were analyzed in 870 participants (additional information on 81 participants not included in publication). Cardiomyopathy-associated genes attributed to metabolic or developmental syndromes were excluded. Variants were graded from class I (benign) to class 5 (pathogenic)

using a modified version of an established scale as adequate data do not exist for the majority of variants to allow for quantitative assessment (Johnston et al., 2012). Variants were filtered on the basis of quality (Most Probable Genotype – MPG ≥10 and MPG/read count of >0.5) and published frequency of the disease prevalence (Appendix G). Variants were designated class 3 (variant of uncertain significance – VUS) if they were: novel missense or in-frame insertion/deletion, missense and in-frame insertion/deletion variants reported a single time as pathogenic without supporting evidence or multiple times with evidence against pathogenicity, and loss of function variants reported once with single evidence against pathogenicity or multiple times with multiple evidence against pathogenicity.

Six hundred and seventy-seven (677) variants were scored class 3 (VUS) because of no publication, or no Human Gene Mutation Database (HGMD)/locus-specific databases (LSDB) entry, or the predicted protein change was in a transcript other than the HGMD reference or single case report without supporting evidence or with conflicting evidence. Participants with class 3 variants were considered for this study. Class 3 variants (VUS) were identified in 36 of the 41 cardiomyopathy-associated genes investigated by Ng et al. Reports of gene-disease association for each gene in HGMD was closely examined. Genes implicated in cardiomyopathy with questionable evidence of pathogenicity were excluded from study. Evidence includes publications with weak disease association and/or functional study, inadequate control data, or disease mechanism other than missense variations. Additionally, genes with fewer than 10 reports on cardiomyopathy and predominantly associated with other phenotypes in HGMD were dropped. Seventeen genes were excluded from the study. The 20 genes selected for the study are listed in Table 3.1.

Table 3.1: List of genes included in study

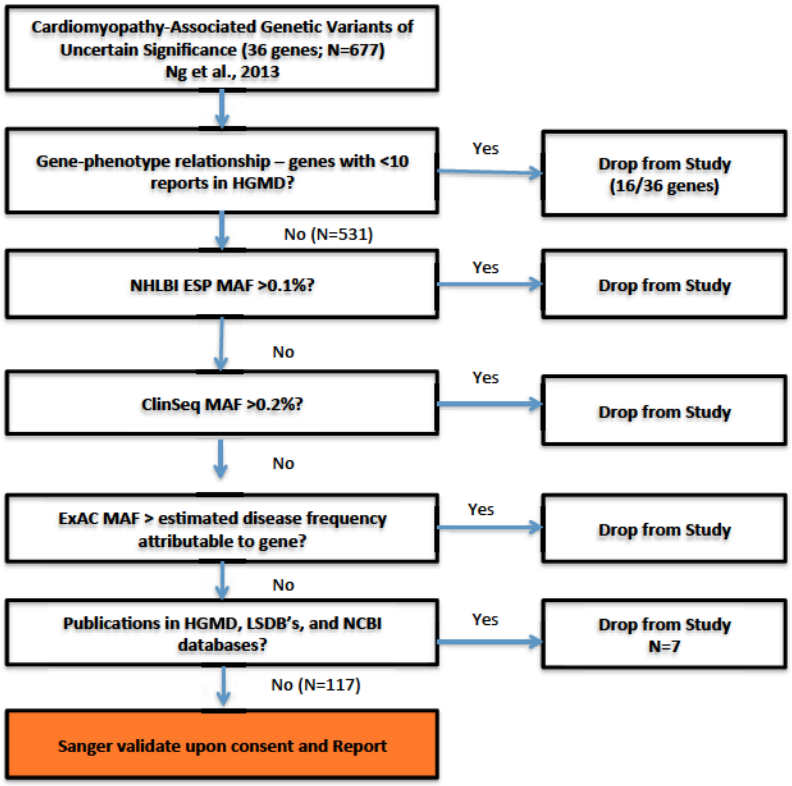
Gene	Cardiomyopathy (# of reports in HGMD)	Other Conditions	Gene	Cardiomyopathy (# of reports in HGMD)	Other Conditions
MYPN (myopalladin)	DCM=12; HCM=1; RCM=1		TPM1 (tropomyosin 1 alpha)	HCM=22; DCM=19; LVNC=5; RCM=1	
PKP2 (plakophillin)	ARVC=193; SCD=5; DCM=1	Brugada syndrome=4	***TMEM43*** (transmenbrane protein 43)	ARVC=10	Emery-Dreifuss muscular dystrophy-related myopathy=2
DSC2 (desmocollin)	ARVC=51; DCM=1; SCD=1		***ACTN2*** (Actinin, alpha 2)	DCM=6; HCM=5	
JUP (junction plakoglobin)	ARVC=18	Cutaneous disease=2; Naxos disease=2; Epidermolysis bullosa=1	***MYL2*** (myosin, light chain 2)	HCM=26; RCM=1	Muscle fibre disease, infantile, type 1=2
DSP (desmoplakin)	ARVC=103; DCM=19	Skin fragility and wooly hair=6; Epidermolysis bullosa=4	***VCL*** (vinculin)	DCM=11; HCM=2; SCD=1	
DSG2 (desmoglein 2)	ARVC=89; DCM=2		***MYH6*** (myosin heavy chain polypeptide 6, cardiac	DCM=9; HCM=4	Congenital heart defects=4; ASD=4; Cardiac dysrhythmia=1
MYBPC3 (myosin binding protein C, cardiac)	HCM=514; DCM=55; LVNC=4; Increased LV wall thickness=2		***BAG3*** (BCL-associated athanogene 3)	DCM=24	Myofibrillar myopathy=4
MYH7 (myosin heavy polypeptide 7, cardiac muscle)	HCM=421; DCM=98; LVNC=23	Myopathy, distal 1=17	***TCAP*** (titin cap)	HCM=6; DCM=5	Muscular dystrophy, limb girdle=5; Intestinal pseudo-obstruction=1
TTN (Titin)	DCM; ARVC	Centronuclear myopathy, tibial muscular dystrophy	***TNNI3*** (troponin I, cardiac)	HCM=53; DCM=10; RCM=10; Increased LV wall thickness=2	
ANKRD1 (ankyrin repeat domain 1, cardiac)	DCM=7; HCM=3	Neurodevelopmental d/o =6; Total anomalous pulmonary venous return=2	***TNNT2*** (troponin T2, cardiac)	HCM=63; DCM=37; RCM=2; Increased LV wall thickness=1	

HCM=hypertrophic cardiomyopathy; DCM=dilated cardiomyopathy; ARVC=arrhythmogenic right ventricular cardiomyopathy; RCM=restrictive cardiomyopathy; SCD=sudden cardiac death

Variant filtering process

Base-calling metrics and filtering for sequence were performed and described by Ng et al (2013). Additionally, variants with allele frequencies of greater than 0.1% in NHLBI's Exome Sequencing Project (ESP), greater than estimated disease prevalence attributable to the gene harboring the variant in the Exome Aggregation Consortium (ExAC) database, and 0.2% in ClinSeq® were excluded, with the assumption that alleles with such frequencies in any population are unlikely to cause a cardiomyopathy with phenotype prevalence of 1/500. Variants with any publications in the Human Gene Mutation Database (HGMD) or LSDB's, or classification other than a VUS in any NCBI databases (ClinVar, dbSNP) were also excluded. The variant assessment process is outlined in Figure 3.1 below.

Figure 3.1: Variant Assessment Process



N= number of variants; MAF = Minor Allele Frequency; NHLBI ESP = National Heart, Lung, & Blood Institute Exome Sequencing Project; LSDB = Locus-specific database; NCBI = National Center for Biotechnology Information

Study Sample

ClinSeq® participants with exome-generated cardiomyopathy-associated variants of uncertain significance (VUS) in one of the 20 genes were consented to participate in this study. One hundred and seventeen (117) variants met the filter criteria. Multiple attempts were made to contact eligible participants of which 81 were successfully consented and 36 were not consented for one of several reasons (requirement to travel to NIH for result -12; result too uncertain – 3; made initial contact but did not return calls or letters – 10; enrolled in on-going ClinSeq® study – 5; deceased – 2; lost to follow-up – 2; withdrawn from ClinSeq® - 2). Of the 81 consented participants that received their VUS results, 79 (97.5%) completed the study survey post disclosure. The demographic characteristics of the study sample are outlined in Chapter 5 (Manuscript 3).

Inclusion/Exclusion Criteria

Inclusion criteria were (a) enrollment in the ClinSeq® study, (b) identified with cardiomyopathy-associated genetic variant of uncertain significance by exome sequencing, and (c) willingness to travel to the NIH to receive result. Exclusion criteria included: (a) inability to validate exome result using Sanger sequencing method (b) interventricular septal wall thickness ≥12mm on baseline echocardiogram, and (c) withdrawal from ClinSeq® project. Participants interested in learning their results but unable to travel to the NIH for reasons of disability or extenuating circumstances were offered the opportunity to learn their outside the study.

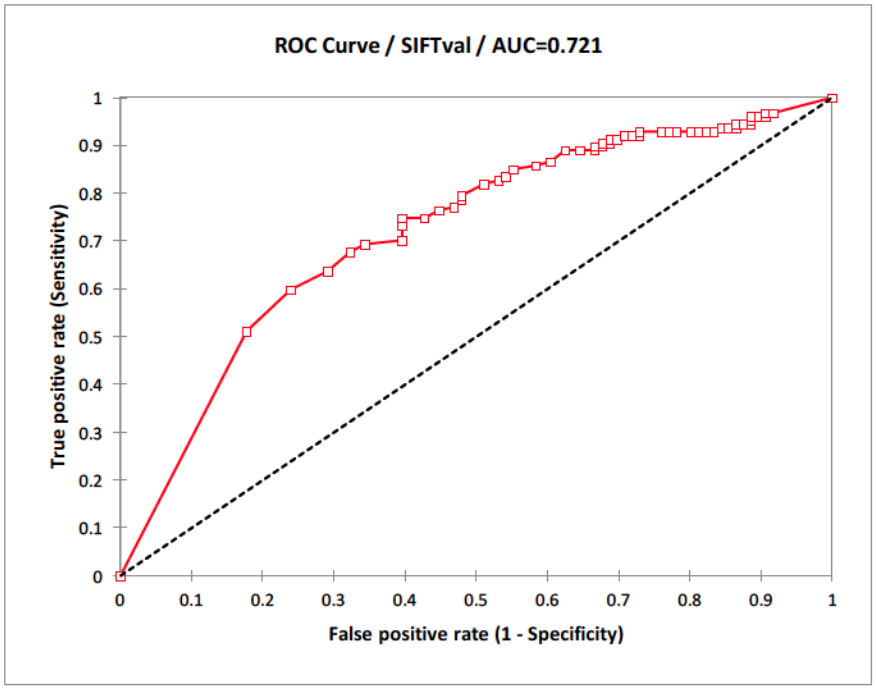
VUS Sub-classification

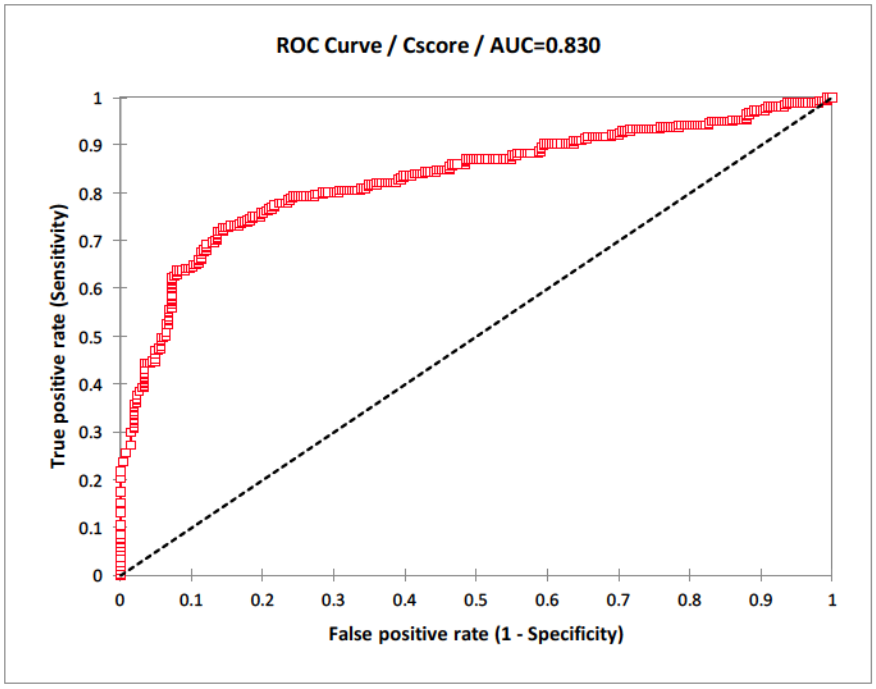
Manually classified genetic VUS's in the 20 genes (see Table 3.1) were analyzed using three widely used general-purpose predictive algorithms: SIFT (sorting intolerant from tolerant) (Kumar, Henikoff, & Ng, 2009); PolyPhen-2 (Adzhubei et al., 2010); and CADD (combined annotation dependent depletion) (Kircher et al., 2014) to determine predicted deleteriousness. We used 2 well-curated data sets of pathogenic and benign variants validated in our laboratory per clinical criteria to define the sensitivities and specificities of the 3 computational tools. Two hundred and fifty-seven (257) manually classified pathogenic variants from various autosomal recessive rare disorders and 264 cardiomyopathy-associated benign variants were used for the relative operating characteristic (ROC) curve comparison. The ROC curve for SIFT and CADD are shown in Figure 3.2. More than half of the PolyPhen-2 output for the cardiomyopathy-associated VUS data set was missing and therefore not included in the ROC curve comparison. The number of observations and the area under the curve (AUC) for CADD (0.830) was higher than those for SIFT (0.721). The ROC analysis for CADD showed a maximum sensitivity and specificity of 72.8% and 85.6% respectively at a C-score of 16.98. SIFT's maximum sensitivity and specificity was 59.8% and 76% respectively.

CADD prediction algorithm was used to determine the deleteriousness of the cardiomyopathy-associated VUS's in the 20 selected genes. C-scores correlated with pathogenicity of both coding and non-coding variants, and based on the rank of each variant relative to all possible 8.6 billion substitutions in the human genome. A C-score ≥10 indicates a variant is predicted to be in the 10% of the most deleterious

substitutions in the human genome, while a score ≥20 indicates a variation in the 1% most deleterious (Kircher et al., 2014). There is no set recommendation for deleteriousness cutoff as any choice would be arbitrary. It is suggested to apply a cutoff on deleteriousness somewhere between 10 and 20 if one is needed. Variants with C-scores ≥ 13.11 were sub-classified as VUS-High (H), and those < 13.11 as VUS-Low (L). The 13.11 C-score cutoff was an attempt to balance the number of variants sub-classified as VUS-H versus VUS-L.

Figure 3.2: ROC curve of two prediction algorithms (SIFT and CADD) analyzing manually classified pathogenic and benign variants.





Procedures

The medical and family histories of eligible participants were reviewed with a medical geneticist familiar with the study prior to consenting and validating their VUS results. Cardiomyopathy-associated VUS results were returned to participants per ClinSeq® protocol: invitation to receive results via phone or mail (Appendices A and B), followed by an in-person meeting with research team, and a written summary report given to participant. Upon obtaining verbal consent of participants' interest in learning results, variants were confirmed using Sanger sequencing in accordance with Clinical Laboratory Improvement Amendments (CLIA) validation regulations. Bi-directional sequencing of PCR amplicons was performed using the Big Dye® Terminator v3.1 sequencing kit and ABI 3130xl DNA Analyzer (Thermo Fisher Scientific Inc., Waltham, MA). Sequences were aligned and compared to the GRCh37 genetic reference to infer homology using the University of California, Santa Cruz (UCSC) Genome Browser (http://genome.ucsc.edu/).

Participants gave written consents prior to disclosure of results. The meaning and ambiguity associated with VUS results were explained along with assurances that declining participation in this study would not affect enrollment in the parent (ClinSeq®) study. Available evidence associated with result classification, location of identified variation within a gene, description of the types of cardiomyopathy and other conditions associated with gene, and prevailing medical recommendations on how to manage cardiomyopathy susceptibility were discussed with participants following a standard protocol (Appendices C and D). Baseline EKG and ECHO findings (septal and

posterior wall thickness, chamber diameter) performed at initial enrollment to the ClinSeq® project were reviewed with participants. The participants advised to follow up with their clinicians if concerned about information received. The participants were offered access to a genetic counselor. If a participant expressed an urgent need for genetic counseling, a genetic counselor with the ClinSeq® study was available to assist with counseling. Recipients that request or are offered counseling were not necessarily removed from study unless he/she decided not to participate. None of the participants requested a genetic counselor during or after the result disclosure sessions. If genetic counseling had been requested, efforts would have been made to ensure that information and recommendations shared with participants are similar.

The average length of the return of result sessions was about an hour. Participants received a copy of the CLIA report with the identified variant at the end of the session. Summary reports containing the gene variant, disease susceptibility, available evidence of gene-disease association, and recommendations regarding disclosed information were mailed to participants within 3 weeks of result disclosure (Appendix F). Recipients were asked to complete a survey to assess their perceived susceptibility (risk), perceived severity, perceived value of received information, self-efficacy, and health behavior intentions 2 weeks post-VUS disclosure (Appendix E). Most participants completed the survey online via a Survey Monkey® account, but 3 participants opted for the paper version. Follow-up e-mail, reminder letters, and phone calls were made by the investigator and a research team member to ensure that participants complete the survey.

Protection of Human Subjects

Potential Risks

Potential risks for the study included psychological distress associated with the uncertainty of receiving genetic information for susceptibility to a potentially life-threatening condition. If distress or significant worry was recognized, the return of result sessions would be stopped and a genetic counselor familiar with the study would provide counseling and offer participant a follow-up visit at the NIH and/or refer them to their primary care provider for further support. A medical geneticist was also available to meet with the participants if needed. Participants were told they could withdraw from this ancillary project at any time. Relatives and offspring of participants could be upset to learn they may be at risk for cardiomyopathy because of the proband's participation in this study. It is possible that participants may seek medical care that could be unnecessary, harmful, or result in significant cost based on learning that they have a VUS that may cause or contribute to a disorder. The Clinical Center will provide short-term medical care for any injury resulting from participation in research at the NIH. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, participants have the right to pursue legal remedy if they believe their injury justifies such action. Finally, participants could have other gene variants associated with cardiomyopathy that may have been missed or not checked for. This could potentially cause some false disease susceptibility reassurance.

Informed Consent

Verbal and written consent were obtained from all participants in this study. The informed consent document was discussed and explained to make clear that participation was completely voluntary and can be stopped at any time for any reason without any impact on participation in the parent study. A private setting was used to discuss the consent process, answer questions, and obtain informed consent.

Adequacy of Protection Against Risks

The study was submitted to the Johns Hopkins Institutional Review Board and the National Human Genome Research Institute Review Board for approval, with subsequent annual IRB renewal obtained.

It is conceivable that contacting participants about their genetic results one to several years after genome sequencing was done could cause emotional distress. To address this concern, participants were asked at the time of enrollment to the parent study if they wished to be offered results in the future. The researcher was prepared to discontinue the return of result sessions if a participant appeared to be in psychological distress, verbally or non-verbally. Survey responses completed online and on paper were assigned custom identifiers unique to each participant. The researcher maintained audio recordings of the result sessions on a secure computer drive.

Possible Benefits

There may or may not be direct benefits to participants; disclosure of results to research participants seeking to learn personal information from genome sequencing provides health information that could be valued by those participants, even when it

may not be actionable or meet standards for clinical utility. Receiving individual research results engages participants with the research study, strengthening the social contract between researchers and participants (Facio et al., 2013). Finally, receiving individual research results increases hypothetical willingness to participate in future studies than increasing compensation or reducing study burden (Kaufman, Murphy, Scott, & Hudson, 2008). No compensation was provided for participation in this study.

Instrumentation

Study Variables and Measures

The survey instruments were selected to optimize information obtained while minimizing participant response burden. All study instruments are listed in the Appendix E. Summary of measurement tools used in this study is shown in Table 3.2.

Table 3.2: Study Measures and Instruments

Variable	Theoretical Definition	Operational Definition	Instrument Description	Psychometrics	Measurement
Perceived Risk	Judgment, attitude, or belief about magnitude of risk with a decision or hazard	ClinSeq Perceived (absolute and comparative) Risk Scale (adapted)	Absolute Risk: 3-item scale with 7-level Likert items from "extremely unlikely" to "extremely likely" Comparative Risk: 3-item scale with 7-level Likert items from "much less likely" to much more likely"	Internal consistency = 0.76 (absolute risk) and 0.86 (comparative risk)	High scores indicative of increased perceived risk
Perceived Severity	Negative consequences, anticipated or current, associated with an event or outcome	Champion's Perceived Severity Scale (adapted)	8-item scale with 5-level Likert items from "strongly disagree" to "strongly agree"	Internal consistency = 0.78; test-retest reliability = 0.76	High scores indicative of increased perceived severity
Perceived Value of Information	Outcome of an evaluative judgment, of the utility of an information or product based on perceptions of what is received	Adapted from ClinSeq Perceived Information Value, Attitudes, Expected Benefits, and Subjective Norms Scales	8-item scale with 5- level Likert items from "strongly disagree" to "strongly agree"	Attitudes: internal consistency = 0.88 to 0.96; Social Norm: internal consistency = 0.82 to 0.93	High scores indicative of increased perceived value of information
Self-efficacy	An individual's conviction about ability to execute an action	2-Item Perceived Self-efficacy Scale	8-item scale with 5- level Likert items from "strongly agree" to "strongly disagree"	--	Low scores indicative of high self-efficacy
Decision Regret	The negative, cognitively-based emotion experienced when realizing or imagining that our present situation would have been better had we acted differently	Decision Regret Scale (DRS)	5-item scale with 5-level Likert items from "strongly agree" to "strongly disagree"	Internal consistency = 0.81 to 0.92; Convergent validity with decision satisfaction (r = -0.40 to -0.60), decisional conflict (r = 0.31 to 0.52)	Total score (0-100) with high scores indicative of high decision regret
Optimism*	Global expectation that more good things than bad will happen in the future	Life Orientation Test –Revised (LOT-R) Scale	3-item scale with 5-level Likert items from "I agree a lot" to "I disagree a lot"	Internal consistency = 0.85	Coded so that high values imply high optimism
Resilience*	Positive adaptation in the face of stress or adversity	Resilience Scale – 14 (RS-14)	14-item scale with 7 potential responses ranging from "disagree" to "agree"	Internal consistency = 0.81	High scores indicative of high resilience
Tolerance of Uncertainty*	Cognitive bias that affects how a person perceives, interprets, and responds to uncertainty	Adapted from Tolerance for Ambiguity (TFA) and Intolerance of Uncertainty (IUS) Scales	7-item scale with 5 potential responses ranging from "not at all characteristic of me" to entirely characteristic of me"	Internal consistency = 0.75	High scores indicative of low tolerance of uncertainty
Health Behavior Intentions	Actions related to decreasing the risk of a certain disease outcome	Adapted from ClinSeq Intentions to Pursue Health Behavior Scale	4-item scale with 5-level Likert items from "definitely no" to "definitely yes"	Internal consistency = 0.58 (VUS results) to 0.90 (preventable conditions)	High scores indicative of intentions to pursue health related behaviors

* Measured at baseline, not measured post result disclosure.

Measures

During the ClinSeq® enrollment visit, participants completed a baseline survey about their perceptions of risk and personal traits such as resilience, dispositional optimism, and tolerance of uncertainty. Descriptions and reliability measures of instruments used in the baseline survey have been reported (Lewis et al., 2015). A committee of experts instrumental in developing the original scales and familiar with the ClinSeq® cohort examined the modified scale for face and content validity prior to instrument administration.

Perceived Risk Scale is a 6-item Likert measure designed to assess absolute and comparative risks associated with a disease or condition. The risk perception scale developed as part of the ClinSeq® baseline survey was adapted for this study. Participants were asked to rate how likely they are to get cardiomyopathy in their lifetime (absolute risk). It also asks participants to rate how likely they are to get cardiomyopathy compared to other people of similar age and sex (comparative risk). Participants were asked to rate their perceived risk on a 7-level Likert scale from 'extremely unlikely' to 'extremely likely' for absolute risk perception (Cronbach's alpha = 0.76), and 'much less likely than the average person' to 'much more likely than the average person' for comparative risk perception (Cronbach's alpha = 0.86). The two sub-scales were combined to measure participants' perceptions of risk associated with cardiomyopathy-associated VUS results. The scale's Cronbach's alpha from this study was 0.84.

The Perceived Severity Scale was adapted from Champions' Perceived Severity Scale, a self-referenced 12-item scale that measures the impact an illness would have on the individual, as well as the severity of the disease itself. The scale also contains items that measure financial security, personal relationships, and emotional response to a disease that are not usually included in measures of severity. For example, respondents are asked to rate the statements "breast cancer is a hopeless disease", "if I got breast cancer, it would be more serious than other diseases", and '"my financial security would be endangered if I got breast cancer". Items are rated on a five-item response scale from "strongly agree" to "strongly disagree" with a Cronbach's alpha score of 0.78 and test-retest reliability of 0.76 across an interval of 2 weeks. These values were observed in a demographic sample similar to the ClinSeq® cohort (majority white, with high school education, high SES) (Champion, 1984). This perceived severity scale was modified to assess the perceived severity of cardiomyopathy susceptibility in VUS recipients 2 weeks post disclosure. The scale's Cronbach's alpha from this study was 0.78.

Perceived Value of Information can be defined as an individual's overall assessment, an outcome of an evaluative judgment, of the utility of a product based on perceptions of what is received and what is given (Sweeney & Soutar, 2001; Zeithaml, 1998). It is a multidimensional construct that is situational and context-specific, and comprised of notions such as quality, benefits, cost, and applicability. The functional value (utility) derived from information received by a research participant will depend on the recipient's perception of acceptable standards of quality, the ability of the information

to enhance recipient's social self-concept, and the feelings or affective state the information generates (emotional value).

The perceived value of information scale used for this study is an 8-item composite scale with 5-level Likert items from "strongly disagree" to "strongly agree". This scale was adapted from 4 ClinSeq® scales – perceived value of information, attitudes (Cronbach's alpha = 0.88 to 0.96), expected benefits (Cronbach's alpha = 0.82 to 0.93), and social norms (Cronbach's alpha = 0.82 to 0.93) (Facio et al., 2013). The scale's Cronbach's alpha from this study was 0.87.

Perceived Self-efficacy was measured with responses to two questions: "I feel confident and competent to pursue health-related behaviors that could help manage and monitor my susceptibility to cardiomyopathy", and "I am certain my efforts to pursue health-related behaviors will be successful". Both questions were rated on a Likert scale of 1 = "very uncertain", to 5 = "very certain".

Self-efficacy is the recipients' conviction that they can successfully execute or perform the recommended health-related behaviors to manage and monitor potential disease susceptibility. Self-efficacy is a good predictor of intention and behavior, and reflects a sense of control over one's environment and behavior. Self-efficacy influences the effort one puts forth to change behavior, and influences the challenges an individual will take on (Bandura, 1997). According to Bandura, self-efficacy should be conceptualized in a situation-specific manner when possible rather than a broad or general sense (Bandura, 1997). Measures of self-efficacy for health-related behaviors refer to beliefs about the ability to perform certain health behaviors, and it is not

necessary to use larger scales if a specific behavior is to be predicted. A rule of thumb for developing perceived self-efficacy item measures is to use the following semantic structure: "I am certain that I can do xx, even if yy (barrier)" (Luszczynska & Schwarzer, 2005). The scale's Cronbach's alpha from this study was 0.89.

The Health Behavior Intentions scale was developed for a previous ClinSeq® study to examine the intentions of participants to receive genetic results that predispose to conditions that are preventable, not preventable, carrier status, and VUS's. Cronbach's alpha scores for the Intention scale ranged from 0.58 (VUS) to 0.90 (preventable disease result), on a 5-level Likert scale from "definitely no" to "definitely yes" (Facio et al., 2013). A modified version of this scale (use of information to change lifestyle/health behaviors; seek additional information about received information; utilize recommended healthcare screening; and share results with valued others) was used to assess intentions.

The intentions of a VUS recipient to act on the disclosed genetic information by changing health behaviors can be partly predicted by their (a) past behaviors when confronted with ambiguous and uncertain information, since past behavior guides future responses and (b) attitudes and subjective norms regarding disclosed information. When behaviors are not well learned or when they are performed in difficult contexts such as the receipt of uncertain genetic information, conscious decision-making is likely to be necessary to carry out the behavior, and a person's intentions are good but imperfect predictors of such behaviors (Ouellette & Wood, 1998). The scale's Cronbach's alpha from this study was 0.83.

Decision Regret Scale (DRS) is a 5-item scale that specifically targets regret associated with health care decisions at a given point in time. It is a well-validated scale with good internal consistency (Cronbach's α = 0.81-0.92) and strong correlation with decision satisfaction (r = - 0.40 to -0.60) and decisional conflict (0.31 to 0.52) (Brehaut et al., 2003). The scale was modified to refer to regret associated with decision to learn VUS results. The 5 items that make up the DRS are scored on a Likert scale from 1 (strongly agree) to 5 (strongly disagree). Items 2 and 4 were reverse-coded so that a higher number indicated more regret. Subtracting 1 from scores for each item and multiplying the resulting number by 25 gave a score ranging from 0 to 100. The items are summed and averaged to obtain a final score; with a score of 0 meaning no regret, scores between 0-30 interpreted as mild regret, and a score of 100 meaning high regret.

Modifying factors such as dispositional optimism, resilience, and tolerance of uncertainty were assessed as part of ClinSeq's baseline survey and not reassessed following disclosure of VUS results.

Dispositional optimism was assessed as the average of three items from the Life Orientation Test –Revised (LOT-R) optimism scale. Participants were asked to rate their responses to: "I'm always optimistic about my future", "In uncertain times, I always expect the best", and "Overall, I expect more good things to happen to me than bad". The Cronbach's alpha score for the scale was calculated to be 0.85. There is a positive correlation of optimism and intentions to change behavior. Optimists are more motivated to obtain positive outcomes than pessimists and use more active than

avoidant coping strategies. Optimists may also be more responsive to the personal utility of health information and therefore more likely to use information provided to make informed decisions (Carver & Scheier, 2014; Aspinwall, Richter, & Hoffman, 2001).

Resilience in ClinSeq® participants, defined as positive adaptation in the face of stress or adversity, was assessed using the 14-item short version of the Resilience Scale (RS) developed by Wagnild and Young (2003). The 25-item RS is the first instrument developed to measure resilience and has been tested in many populations of normal and clinical samples. Cronbach's alpha coefficients range from 0.72 – 0.94 for the 25-item RS, and 0.81 for the 14-item short version RS, supporting the internal consistency reliability of the Resilience Scale (Wagnild & Young, 2003; Wagnild, 2009). At baseline, ClinSeq® participants were asked to rate how strongly they agree with statements such as: "I usually manage one way or another", I can get through difficult times because I have experienced difficulty before", and "I usually find something to laugh about". Tolerance of uncertainty was assessed using a 7-item tolerance for ambiguity (TFA) scale with an acceptable internal reliability (Cronbach's alpha = 0.75) (Geller, Tambor, Chase, & Holtzman, 1993). Participants were asked to rate their response from 1 "not at all characteristic of me" to 5 "entirely characteristic of me" to statements such as: "it disturbs me when I am unable to follow another person's train of thought", "if I am uncertain about the responsibilities involved in a particular task, I get very anxious", and "before any important task, I must know how long it will take".

Statistical Analyses

All analyses for this study were accomplished using STATA 14 (StataCorp. College Station, Texas). Data analysis began with descriptive and exploratory statistical analyses. Study variables were examined to assess distributions, and to note outliers or extreme observations. Bivariate correlations were examined for variables predicted to describe health behavior intentions. Specifically, the variables of perceived risk, perceived severity, perceived information value, self-efficacy, and intentions were assessed.

Aim 1: To describe the effect of perceived risk and perceived severity associated with disclosure of genetic variants of uncertain significance results on health behavior intentions.

Hypothesis $_1$: High levels of perceived risk and severity lead to high intentions to pursue health related behaviors.

To test hypothesis 1, the following steps were taken. The individual effects of perceived risk and perceived severity on health behavior intentions were examined in bivariate analyses. Correlation analyses were conducted, applying Pearson's correlation coefficient to examine the association of perceived risk/perceived severity to health behavior intentions. The extent that health behavior intentions could be considered a function of perceived risk and perceived severity was assessed through multiple linear regression. To address potential confounders, the following steps were taken:

a) Participant characteristics associated with high levels of health behavior intentions (significance level $\alpha = 0.05$) were identified.

b) The relation of these variables to perceived risk and perceived severity were assessed.

c) A variable was considered a potential confounder in subsequent multivariable models if there was a relationship of the variable to perceived risk and perceived severity, as well as a relationship of that variable to health behavior intentions which was not in the causal pathway between perceived risk /perceived severity and health behavior intentions.

Aim 2: Examine the effect of perceived value of information and self-efficacy associated with disclosure of genetic variants of uncertain significance results on health behavior intentions.

Hypothesis $_2$: High levels of perceived information value and self-efficacy increase intentions to pursue health related behaviors.

Similar to the analysis for Aim 1, the following steps were taken. Individual effects of perceived value of information and self-efficacy on health behavior intentions were examined in bivariate analyses. Correlations were examined to assess the association of perceived value of information, self-efficacy to health behavior intentions. The extent to which the level of health behavior intentions could be considered a function of

perceived value of information and self-efficacy was assessed through multiple linear regressions. To address potential confounders, the following steps were taken:

a) Participant characteristics associated with high levels of health behavior intentions were identified.

b) Relationships between these characteristics and the variables of perceived value of information and self-efficacy were assessed.

c) A variable was considered as a potential confounder in subsequent multivariate model if there was a relation of a variable to perceived value of information or self-efficacy, and a relation of that variable to health behavior intentions which was not in the causal pathway between perceived value of information or self-efficacy and health behavior intentions.

Aim 3: Examine the influence of resilience, optimism, and tolerance of uncertainty on health behavior intentions post disclosure of genetic variants of uncertain significance results.

Hypothesis $_3$: Resilience, optimism, and tolerance of uncertainty have a moderating influence on intentions to pursue health related behaviors.

The following steps were taken to examine the moderating influence of resilience, optimism, and tolerance of uncertainty on participants' intentions to pursue health related behaviors. Moderating effects of these variables are indicated by the

interaction of each variable with the predictor variables (perceived risk, perceived severity, perceived value of information, and self-efficacy) in explaining health behavior intentions. A moderator variable is a variable that alters the direction or strength of relationship among variables. Ideally, the moderator variable should be measured before the predictor variables, as in this study. The moderation effects of resilience, optimism, and tolerance of uncertainty were tested using multiple regression analysis. The regression coefficients for the interaction terms provide an estimate of the interaction effect on the outcome variable (Fairchild & MacKinnon, 2009). The level of significance of $\alpha = 0.05$ was used.

Aim 4: Measure the level of regret associated with decision to learn genetic variants of uncertain significance results 2 weeks post disclosure.

Hypothesis $_4$: The uncertainty associated with genetic VUS information increases regret with decision to learn result.

To test hypothesis 4, the following steps were taken. The DRS was used to measure participants' level of regret with decision to learn their VUS results. Correlation analysis was used to determine the strength of relationship between decision regret and the sub-classification of VUS.

Finally, before multiple linear regressions analyses, two important steps were performed. First, each study variable was examined in relation to health behavior intentions through a series of histograms and scatterplots. Second, collinearity

diagnostics were employed to ascertain if multicollinearity was a valid concern. Multicollinearity (near perfect linear relationships among predictor variables) is a concern because as it increases, the regression model coefficients become unstable and the standard errors of the coefficients can be inflated (http://www.ats.ucla.edu/stat/sas/notes2/). The statistical parameter used to check for multicollinearity was the variance-inflation factor (VIF). The VIF is indicative of the degree to which the precision of the model (R^2) is degraded by multicollinearity. VIF values greater than 10 may merit further investigation, as the variable could be considered a linear combination of other independent variables already in the regression equation (Fox, 1984).

References

Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E. Gerasimova, A., Bork, P., et al. (2010). *Nat Methods*, **7**(4), 248-249.

Aspinwall, L.G., Richter, L., Hoffman, III R.R., Chang, E.C. (Ed). (2001). *Optimism & pessimism: implications for theory, research, and practice*, (pp. 217-238). Washington, DC. US: American Psychological Association.

Azjen, I. (1991). The theory of planned behavior. *Organizational Behavior and Human Decision Processes,* **50**, 179-211.

Bandura, A. (1997). Self-efficacy: The Exercise of Control. New York: W.H. Freeman.

Biesecker, L.G., Mullikin, J.C., Facio, F.M., Turner, C., Cherukuri, P.F., Blakesley, R.W., et al. (2009). The ClinSeq Project: piloting large-scale genome sequencing for research in genomic medicine. *Genome Res,* **19**(9), 1665-74.

Brehaut, J.C., O'Connor, A.M., Wood, T.J., et al. (2003). Validation of a decision regret scale. *Med Decis Making*, 23,281-92.

Carver, C.S., & Scheier, M.F. (2014). Dispositional optimism. *Trends Cogn Sci,* 18(6), 293-9.

Champion, V.L. (1984). Instrument development for health belief model constructs. *Advanced Nursing Science,* **6**(3), 73-85.

Facio, F.M., Eidem, H., Fisher, T., et al. (2013). Intentions to receive individual results from whole-genome sequencing among participants in the ClinSeq study. *Eur J Hum Genet*, 21(3), 261-265.

Fairchild, A.J., & MacKinnon, D.P. (2009). A general model for testing mediation and moderation effects. *Prev Sci,* 10(2), 87-99.

Ferriere, M., & Van Ness, B. (2012). Return of individual research results and incidental findings in the clinical trials cooperative group setting. *Genet Med,* **14**, 411-416.

Frank, T.S., Deffenbaugh, A.M., Reid, J.E., Hulick, M., Ward, B.E., Lingenfelter, B., Gumpper, K.L., et al. (2002). Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. *J Clin Oncol,* **20**, 1480-1490.

Fox, J. (1984). *Linear statistical models and related methods: With applications to social research*. New York: John Wiley.

Geller, G., Tambor, E.S., Chase, G.A., Holtzman, N.A. (1993). Measuring physicians' tolerance for ambiguity and its relationship to their reported practices regarding genetic testing. *Medical Care,* 31(11), 989-1001.

Introduction to SAS. UCLA: Statistical Consulting Group. from http://www.ats.ucla.edu/stat/sas/notes2/ (accessed October 20, 2016).

Johnston, J.J., Rubinstein, W.S., Facio, F.M., Ng, D., Singh, L.N., Teer, J.K., et al. (2012). Secondary variants in individuals undergoing exome sequencing: screening of 572 individuals identifies high-penetrance mutations in cancer-susceptibility genes. *Am J Hum Genet,* **91**, 97–108.

Kaufman, D., Murphy, J., Scott, J., Hudson, K. (2008). Subjects matter: a survey of public opinions about a large genetic cohort study. *Genet Med,* 10(11), 831-839.

Kircher, M., Witten, D.M., Jain, P., O'Roak, B.J., Cooper, G.M., Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet*, Feb 2. doi: 10.1038/ng.2892.

Kumar, P., Henikoff, S., & Ng, P.C. (2009). Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc,* 4(7), 1073-81.

Lewis, K.L., Han, P.K., Hooker, G.W., Klein, W.M., Biesecker, L.G., Biesecker, B.B. (2015). Characterizing participants in the ClinSeq genome sequencing cohort as early adopters of a new health technology. *PloS One* DOI: 10.1371/journal.pone.0132690.

Luszczynska, A., & Schwarzer, R. (2005). Multidimensional health locus of control: Comments on the construct and its management. *Journal of Health Psychology*, 10, 633-642.

Ouellette, J.A., & Wood, W. (1998). Habit and intention in everyday life: The multiple processes by which past behavior predicts future behavior. *Psychological Bulletin,* **124**(1), 54-74.

Plon, S.E., Eccles, D.M., Easton, D., Foulkes, W.D., Genuardi, M., Greenblatt, M.S., et al. (2008). Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum. Mutat,* **29**, 1282–1291

StataCorp. (2015). *Stata* Statistical Software: Release *14*. College Station, TX: Stata Press.

Sweeney, J.C., & Soutar, G.N. (2001). Consumer Perceived Value: The development of a

multiple item scale. *Journal of Retailing,* **77**(2), 203–20.

Wagnild, G.M., & Young, H.M. (1993). Development and psychometric evaluation of the Resilience Scale. *J Nursing Measurement,* 1(2), 165-78.

Wagnild, G.M. (2009). A review of the Resilience Scale. *J Nursing Measurement,* 17(2), 105-13.

Wolf, S.M., Crock, B.N., Van Ness, B., Lawrenz, F., et al. (2012). Managing incidental findings and research results in genomic research involving biobanks and archived data sets. *Genet Med,* **14**, 361-384.

Zeithaml, V.A. (1988). Consumer perceptions of price, quality and value: A means-end model and synthesis of evidence. *Journal of Marketing, 52*(7), 2–22.

CHAPTER FOUR: MANUSCRIPT TWO

Title: Regret associated with decision to learn uncertain genetic results.

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Abstract

Purpose: This study examined how decisions to learn genetic variants of uncertain significance results affect regret following disclosure.

Methods: Participants from the ClinSeq® with cardiomyopathy-associated variants of uncertain significance were given the choice to receive their genetic results. The level of decision regret associated with their result was measured 2 weeks post disclosure.

Results: Sixty-eight (68) participants (44.1% female; 83.8% White; mean age = 63) were included in our analysis. The mean decision regret score was 12.41 (SD=16.42) on a 0 to 100 scale (score of 0 = no regret). Thirty-five participants (51.5%) reported no regret (0/100) associated with their decision, and 57 (83.8%) reported a score of 30/100 or less. Sub-classification of VUS results into high and low groups showed moderate significant correlation ($r = -0.26$, $p = 0.035$) with regret post disclosure.

Conclusions: Despite the ambiguity associated with genetic variants of uncertain significance, there is minimal regret regarding a decision to learn these results.

Key Words: genetic result disclosure; variants of uncertain significance; decision-making; decision regret.

Introduction

Genomic information and its associated uncertainty is increasingly becoming a part of decision-making in health care as knowledge and interest in genetics grows (Schulte, Rothaus, Adler, & Phimister, 2014; Cooper, 2015). How an individual perceives uncertainty likely predicts decision to learn sequencing results and to act on the information (Biesecker et al., 2014). There is limited evidence to predict how patients and research participants respond to uncertainties associated with genomic sequencing information (Han, Klein, & Arora, 2011). Uncertainty in genomics stems from the probability, ambiguity, and complexity inherent to the information. Probability (or risk) expresses the indeterminacy of future outcomes; ambiguity describes when the information or evidence is imprecise; and complexity refers to features of available information that makes it difficult to understand (Han, Klein, & Arora, 2011).

Variants of uncertain significance (VUS) are alterations in the DNA sequence of a gene that have uncertain effects on the function of the gene product or disease risk. As next generation sequencing becomes an increasingly important approach for evaluating disease risk, it is also identifying VUS in a higher proportion compared to single gene testing (Maxwell et al., 2014; Kurian et al., 2014). There are no commonly accepted guidelines for providing clinical interpretation and recommendations for those receiving VUS results, therefore such findings can be challenging for patients and health care providers in research and clinical genetics settings (Culver et al., 2013; Vos et al., 2008).

The growing efforts to make health care more patient-centered is driving the need to help researchers and clinicians communicate the uncertainties of genetic results

amidst the limited understanding and fragmented body of knowledge available on VUS interpretations (Han, Klein, & Arora, 2011). As patients become more involved in their health care and share decision-making with healthcare providers, negative experiences surrounding their decisions can lead to regret (Brehaut et al., 2003). Regret is the adverse emotion one experiences with the realization that the current situation would be more favorable if one had chosen differently (Zeelenberg & Pieters, 2007). A key goal of decision making is to minimize regret. Although regret is a common consequence of decisions, it is an effective indicator for assessing the quality of information an individual used to arrive at a decision (Joseph-Williams, Edwards, & Elwyn, 2011; Carere et al., 2016), satisfaction with medical decision-making, and experience with the health care system (Clark, Wray, & Ashton, 2001). Nonhypothetical research examining decision regret associated with genetic results is pivotal to developing decision aids that help patients with genetics-related decision conflicts (Stacey et al., 2014; Becerra Perez, Menear, Brehaut, & Legare, 2016).

Decision-making, especially in the face of uncertain or equivocal evidence, is sensitive to personal preferences (Elwyn & Miron-Shatz, 2010). Communication of uncertainty regarding disease risk estimates has complex effects including distress consistent with ambiguity aversion and distortion of recipients' risk perception. These responses are influenced by the recipients' personality type, and perceived credibility and value of disclosed information. Although uncertain information may generate anxiety and distress, it can also be autonomy-promoting (Han, 2013).

Here, participants enrolled in a longitudinal genetic study were given the choice of learning genetic results classified as VUS. We aimed to explore the level of decision regret associated with recipients' decision to learn genetic information with limited evidence and unknown outcomes. This was conducted as part of a broader project exploring the impact of VUS results on health-related behaviors.

Materials and Methods

Study Design

Eligible participants were recruited from the ClinSeq project, an exome sequencing study designed to pilot the application of clinical exome and genome sequencing. ClinSeq participants were consented to receive genetic results that predispose to preventable or treatable conditions, unpreventable or untreatable conditions, those that establish carrier status for a condition, and those of uncertain clinical significance (Biesecker, Mullikin, Facio, et al., 2009). In a previous ClinSeq project, exome data from 41 cardiomyopathy- and 22 cardiac arrhythmia-associated genes in 870 participants were analyzed using an algorithm that filtered results on genotype quality, frequency, and primary literature identified from genetic databases. A total of 1367 variants were identified in the 41 cardiomyopathy-associated genes, of which 677 were classified as VUS. Participants were not selected for a history of arrhythmia, cardiomyopathy, or family history of sudden cardiac death (Ng et al., 2013).

For the present study, the 677 cardiomyopathy-associated variants were further filtered based on: quality of evidence associating a gene with cardiomyopathy; allele

frequency greater than the estimated cardiomyopathy disease prevalence of 1/500 in the ClinSeq® cohort and publicly available genetic databases; and when available, allele frequency greater than the estimated disease prevalence attributable to a specific gene in any population in the Exome Aggregation Consortium (ExAC) database (Lek et al., 2016).

Participants gave verbal consent by phone regarding their interest in learning their result. Results were validated in a Clinical Laboratory Improvement Act (CLIA)-compliant process and disclosed during an in-person NIH Clinical Center 1-hour audiotaped session by a research nurse, with a medical geneticist and genetic counselor available if needed or requested. Participants gave written consent to learn their results and permission to audiotape their session. The return of result session provided information on: what it means for a variant to be classified as having uncertain significance, the gene in which the variant was identified and its basic functions, conditions associated with mutations in the gene, basic genetics and inheritance of the result, and recommendations if concerned about disclosed information.

A predictive algorithm (Combined Annotation Dependent Depletion - CADD, Kircher, Witten, Jain et al., 2014) was used to divide the cardiomyopathy-associated genetic VUSs into two groups (VUS-High and VUS-Low). CADD is a tool for scoring the deleteriousness of single nucleotide variants that integrates multiple annotations into one metric (C-score). C-scores strongly correlate with pathogenicity of coding and non-coding variants (Kircher, Witten, Jain et al., 2014). Participants were told which group their result was placed and that this sub-classification was solely for research purposes

and not to inform or guide clinical decisions, and this sub-classification was not included in the written test report.

Participants received a copy of their CLIA-certified variant report at the end of the session and were sent a letter summarizing their result and our recommendation within 2 weeks of their visit. Participants were also asked to complete an online survey in the summary letter.

A modified version of the Decision Regret Scale (DRS) was used to assess the level of regret recipients associated with the decision to learn their VUS result (Table 4.1).

Table 4.1: Decision Regret Scale for Decision to Learn VUS Result

Please think about the decision you made about receiving this genetic VUS result. Please show how strongly you agree or disagree with these statements by circling a number from 1 (strongly agree) to 5 (strongly disagree) that best fits your views about your decision.

It was the right decision	1 Strongly agree	2 Agree	3 Neither agree nor disagree	4 Disagree	5 Strongly disagree
I regret the choice that was made	1 Strongly agree	2 Agree	3 Neither agree nor disagree	4 Disagree	5 Strongly disagree
I would go for the same choice if I had to do it over again	1 Strongly agree	2 Agree	3 Neither agree nor disagree	4 Disagree	5 Strongly disagree
The choice did me a lot of harm	1 Strongly agree	2 Agree	3 Neither agree nor disagree	4 Disagree	5 Strongly disagree
The choice was a wise one	1 Strongly agree	2 Agree	3 Neither agree nor disagree	4 Disagree	5 Strongly disagree

The DRS is a 5-item scale that specifically targets regret associated with health care decisions at a given point in time. It is a well-validated scale with good internal consistency (Cronbach's α = 0.81-0.92) and strong correlation with decision satisfaction (r = - 0.40 to -0.60) and decisional conflict (0.31 to 0.52) (Brehaut, O'Connor, Wood et al., 2003). The scale was modified to refer to regret associated with decision to learn VUS results. The summary letter described the nature of the result they received, the

gene in which the variant was identified, description of conditions previously associated with mutations in the gene, pattern of genetic inheritance associated with each condition, VUS sub-classification based on a predictive algorithm, and our recommendations were the participants to be concerned about developing any of the listed conditions.

The Johns Hopkins University and National Human Genome Research Institute's institutional review boards approved the study. Participants were not compensated for their participation in the ClinSeq study or this ancillary project.

Data Analysis

The 5 items that make up the DRS are scored on a Likert scale from 1 (strongly agree) to 5 (strongly disagree). Items 2 and 4 were reverse-coded so that a higher number indicated more regret. Subtracting 1 from scores for each item and multiplying the resulting number by 25 gave a score ranging from 0 to 100. The items are summed and averaged to obtain a final score; with a score of 0 meaning no regret, scores between 0-30 interpreted as mild regret, and a score of 100 meaning high regret. Correlation analysis was used to determine the strength of relationship between decision regret and the sub-classification of the VUS.

Results

Participants

A total of 68 participants completed the DRS as part of the 2-week post disclosure survey. Multiple attempts (phone calls and letters) were made to contact participants that did not complete survey at the 2-week time point. Demographic characteristics of the 68 participants included in this study are presented in Table 4.2.

Table 4.2: Demographics Characteristics (n=68)

Characteristic	Participant, n (%)
Race/Ethnicity	
White	57(83.8)
Asian and not Hispanic or Latino	6(8.8)
Hispanic or Latino	1(1.5)
African-American	3(4.4)
Other	1(1.5)
Education	
High School	2(2.9)
Some college/Technical school	8(11.8)
College	24(35.3)
Postgraduate	32(47.1)
Not reported	2(2.9)
Age (years)	
50-65	42(61.8)
66-75	26(38.2)
Sex	
Female	30(44.1)
VUS	
Low	33(48.5)
High	35(51.5)
Bins	
1-3	53(77.9)
4	15(22.1)

VUS = Variant of Uncertain Significance; sub-classified into Low and High
Bins = 10-year Framingham risk: 1(<5%); 2(5%-10%); 3(>10%); 4(known coronary artery disease)

Most participants were white and had at least a college education; they ranged in age from 52 to 75 years (Table1). These demographic characteristics are consistent with those of larger samples of the ClinSeq population (Facio, Brooks, Loewenstein, et al., 2011)

Decision Regret

The measure of regret associated with decision to learn VUS results is displayed in Figure 4.1.

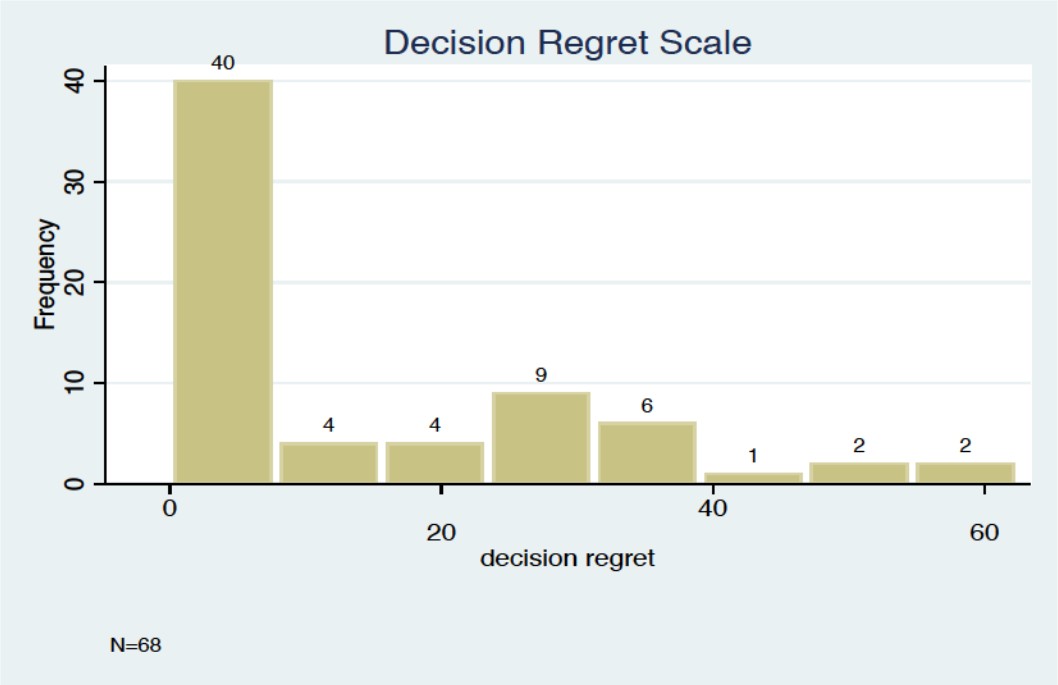

Figure 4.1: Scores for decision regret

With a mean of 12.40 (SD = 16.42), the scores were skewed towards no regret, with 51.5% of participants reporting no regret (score= 0), and 83.8% reporting no or mild regret (scores= 0-30). There was a statistically significant moderate correlation between decision regret and the sub-classes of VUS. Participants in the VUS-Low group reported higher regret scores on decision to learn their VUS results compared to those in the VUS-High group (r= -0.256, p= 0.035) (Figure 4.2). The highest regret score of 62.5 was recorded in the VUS-Low group.

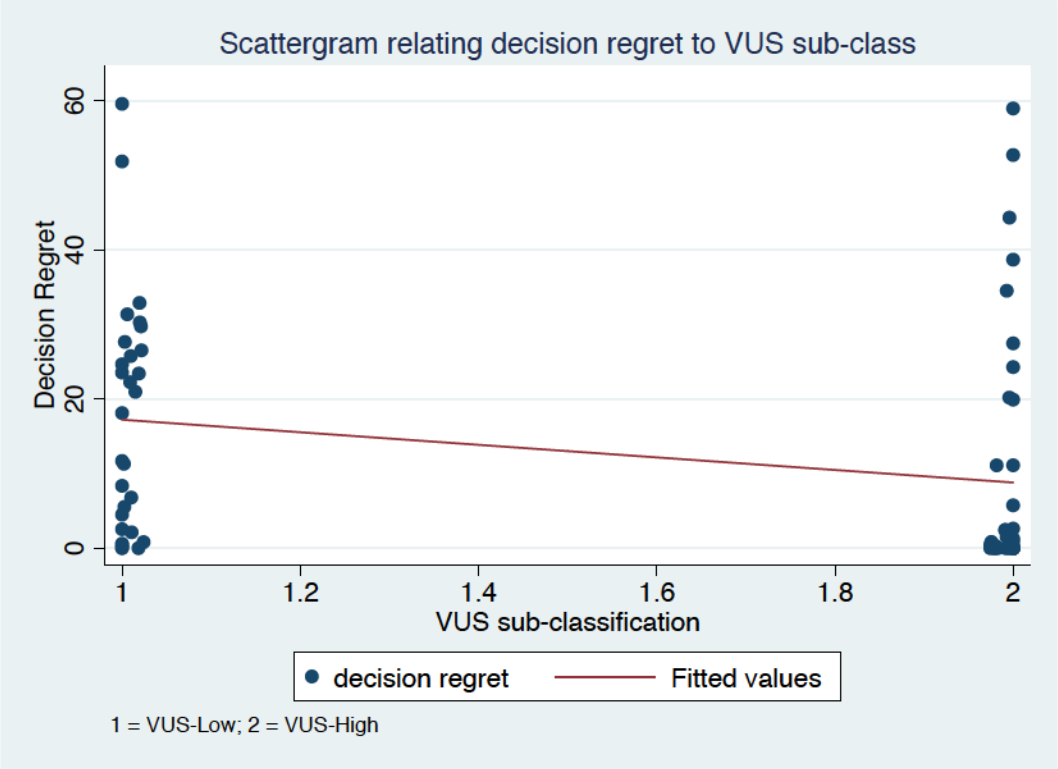

Figure 4.2: Decision regret versus VUS sub-classification

Discussion

Participants in this study experienced little to no regret regarding their decision to learn their VUS results. The sub-classification of VUS results into high and low subcategories did lead to a modestly greater (but still low) level of regret in the VUS-Low group, compared to VUS-High.

There are four potential explanations for our findings. First, participants typically enroll in a genetic study to learn information not easily discerned from personal or family history, the decision to learn genetic results with such uncertainty and no standard recommendations could be seen as informative and having some yet to be understood significance. Receipt of a VUS result may have increased participants' self-awareness of health matters, especially personal or familial heart issues beyond cardiomyopathy. Additionally, learning their VUS results could have satisfied a curiosity or interest, even if no strong health implication was attached to the disclosed information (Lewis, Hooker, Connors, et al., 2015). This may have reduced participants' potential for regret.

Second, decision regret was measured 2 weeks post result disclosure with a mean score of 12.40 (SD=16.43). High mean scores have been reported in studies assessing regret months to years after decision is made (Becerra Perez, Menear, Brehaut, et al., 2016), but the aspects of regret (process, option or outcome) measured over this length of time is unclear. The DRS uses two definitions of regret; one focusing on option, the other on outcome (Joseph-William, Edwards, & Elwyn, 2011). Although they can be experienced independently or in combination, measuring regret soon after

a decision is made focuses mainly on option regret. Additionally, post-decision cognitive processes may reduce feelings of regret over time (Brehaut, O'Connor, Wood, et al., 2003).

Third, this was a binary decision – learn or decline results. It is possible that study participants anticipated the regret of not learning results and chose to minimize the negative emotion by choosing to learn results. It is also plausible that previously experienced regret with declining medical results influenced their decision-making (Zeelenberg & Beattie, 1997).

Lastly, an average of 50 minutes was spent with each participant explaining the meaning of a VUS, describing the function of the gene with the alteration, conditions associated with alterations in the cardiomyopathy-associated gene, description of cardiomyopathy and other conditions associated with the gene, mode of genetic inheritance, and recommendations if concerned about information received. This extensive clinical encounter did not factor into decision to learn their VUS results, but post disclosure it could have mitigated some decision regret.

The pathogenicity probability for VUS classification ranges from greater than 5% to less than 90% (Richards et al., 2015). Our sub-classification of VUS results into VUS-High and Low was an attempt to determine whether recipients' perceptions of risk and utility would be different based on their sub-group. Our findings show that participants had different responses to these two types of results. This suggests that participants can receive and process genetic information with varying degrees of uncertainty. It is possible that the perceptions of risk and information utility of participants in the VUS-

Low group was tempered following result disclosure, leading to regret about decision to learn results compared to those in the VUS-High group. The perceived susceptibility to cardiomyopathy for the VUS-Low group may be low post disclosure if the initial decision of participants to learn their VUS results was to prevent or reduce the risk of disease. Therefore, the time invested in learning their VUS-Low results (NIH visit, about an hour with the investigator, survey completion) could be perceived as not worthwhile. Similar findings have been reported in regret associated with cancer treatment and body image (Gahm, Wickman, & Brandberg, 2010; Spittler, Pallikathayil, & Bott, 2012).

Decision regret is a key patient reported outcome (Oliver & Greenberg, 2009), and is an increasingly accepted measure of good decision-making. Decision regret scale (DRS) is validated to measure regret associated with actual, and not anticipated, health related decisions (Joseph-Williams, Edwards, & Elwyn, 2011). Some data suggest that return of individual results to participants may engender negative emotional responses (Manolio et al., 2013; Yu et al., 2014). However, other data show that adverse psychological outcomes (Bloss, Schork, & Topol, 2011), and specifically high decision regret in recipients of genetic results (Carere et al., 2016) occur in fewer than 3% of cases.

The type of health-related decision (treatment, screening, or prevention) that is confronted by an individual influences decision regret, with increased regret associated with treatment decisions, when compared to screening or disease prevention decisions (Becerra Perez et al., 2016). The decision of participants to learn of their cardiomyopathy-associated VUS, and our recommendation that they share the results

with health care providers if they were concerned about the risk of developing any of the conditions discussed is not a treatment decision in itself. Based on personal and family history, sharing uncertain genetic information with one's clinician could potentially lead to non-invasive tests such as echocardiography, electrocardiography, or cardiac-specific blood work.

This study has some limitations. First, participants were predominantly white, well-educated, high income, and older than reproductive age individuals. Decision regret seems to be a general human phenomenon across clinical contexts that do not appear to be related to demographics or specific groups (Becerra Perez et al., 2016). There is potential for selection bias as our participants are early adopters of sequencing technology (Lewis et al., 2015), therefore the findings on regret associated with decision to learn VUS results may not fully describe the experiences of others faced with similar decisions. Second, regret was not measured at multiple time points to capture potential regret associated with later outcome of the decision to learn VUS results. There is a temporal aspect to regret that was not measured by this cross-sectional study. Reported regret 2 weeks post disclosure may not fully represent a participant's experience over time.

Conclusions

Regret is a powerful emotion and a common consequence of decision-making. Decision regret within the context of the uncertainty inherent to genomic information is poorly understood. Insight into the consequences of decision-making will be useful as patients and research participants become involved in the management of their genetic results.

This study shows that research participants who receive VUS results have little regret regarding the decision in spite of the uncertainty associated with the disclosed information. These data suggest that groups receiving VUS-High had a different level of regret than did those with VUS-Low. This may in turn suggest that such sub-classification efforts may lead to categories of results that are distinguishable, and possibly inform future standards of returning sequencing information to research participants and patients.

References

Biesecker, B.B., Klein, W., Lewis, K.L., Fisher, T.C., Wright, M.F., Biesecker, L.G., et al. (2014). How do research participants perceive "uncertainty" in genome sequencing? *Genet Med*, 16, 977-80.

Biesecker, L.G., Mullikin, J.C., Facio, F.M., Turner, C., Cherukuri, P.F., Blakesley, R.W., et al: NISC Comparative Sequencing Program. (2009). The ClinSeq Project: piloting large-scale genome sequencing for research in genomic medicine. *Genome Res,* 19, 1665-1674.

Bloss, C.S., Schork, N.J., & Topol, E.J. (2011). Effect of direct-to-consumer genomewide profiling to assess disease risk. *N Engl J Med*, 364, 524-534.

Brehaut, J.C., O'Connor, A.M., Wood, T.J., Hack, T.F., Siminoff, L., Gordon, E., et al. (2003). Validation of a decision regret scale. *Med Decis Making*, 23, 281-92.

Becerra Perez, M.M., Menear, M., Brehaut, J.C., & Legare, F. (2016). Extent and predictors of decision regret about health care decisions: a systematic review. *Med Decis Making*, 36(6), 777-90.

Carere, D.A., Kraft, P., Kaphingst, K.A., Roberts, J.S., & Green, R.C. (2016). Consumers report lower confidence in their genetics knowledge following direct-to-consumer personal genomic testing. *Genet Med*, 18(1), 65-72

Clark, J.A., Wray, N.P., & Ashton, C.M. (2001). Living with treatment decisions: regrets and quality of life among men treated for metastatic prostate cancer. *J Clin Oncol,* 19(1), 72-80.

Cooper, G.M. (2015). Parlez-vous VUS? *Genome Res*, 25(10), 1423-6.

Culver, J., Brinkerhoff, C., Clague, J., Yang, K., Singh, K., Sand, S., et al. (2013). Variants of uncertain significance in BRCA testing: evaluation of surgical decisions, risk perception, and cancer distress. *Clinical Genetics, 17*(10), 12097. doi:10.1111/cge.

Elwyn, G., & Miron-Shatz, T. (2010). Deliberation before determination: the definition and evaluation of good decision making. *Health Expect,* 13(2), 139-47.

Facio, F.M., Brooks, S., Loewenstein, J., Green, S., Biesecker, L.G., & Biesecker, B.B. (2011). Motivators for participation in a whole-genome sequencing study: implications for translational genomics research. *Eur J Hum Genet*, 19, 1213-1217.

Gahm, J., Wickman, M., & Brandberg, Y. (2010). Bilateral prophylactic mastectomy in women with inherited risk of breast cancer: prevalence of pain and discomfort, impact on sexuality, quality of life and feelings of regret two years after surgery. *Breast,* 19, 462-9

Han, P.K. (2013). Conceptual, methodological, and ethical problems in communicating uncertainty in clinical evidence. Med Care Res Rev, 70(1 Suppl),14S–36S.

Han, P.K., Klein, W.M., & Arora, N.K. (2011). Varieties of uncertainty in health care: a conceptual taxonomy. *Med Decis Making,* 31, 828-838.

Joseph-Williams, N., Edwards, A., & Elwyn, G. (2011). The importance and complexity of regret in the measurement of 'good' decisions: a systematic review and content analysis of existing assessment instruments. *Health Expect,* 14, 59-83.

Kircher, M., Witten, D.M., Jain, P., O'Roak, B.J., Cooper, G.M., & Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet*, Feb 2. doi: 10.1038/ng.2892.

Kurian, A. W., Hare, E. E., Mills, M. A., Kingham, K. E., McPherson, L., Whittemore, A., et al. (2014). Clinical evaluation of a multiple-Gene sequencing panel for hereditary cancer risk assessment. *Journal of Clinical Oncology, 14*, 14.Google Scholar

Lek, M., Karczewski, K.J., Minikel, E.V., Samocha, K.E., Banks, E., Fennell, T., et al. (2016). Analysis of protein-coding genetic variation in 60,706 humans. *Nature,* 17, 285-91.

Lewis, K.L., Hooker, G.W., Connors, P.D., Hyams, T.C., Wright, M.F., Caldwell, S., et al. (2016). Participant use and communication of findings from exome sequencing: a mixed-methods study. *Genet Med*, 18(6), 577-83.

Manolio, T.A., Chisholm, R.L., Ozenberger, B., et al. (2013). Implementing genomic medicine in the clinic: the future is here. *Genet Med*, 15, 258-267

Maxwell, K. N., Wubbenhorst, B., D'Andrea, K., Garman, B., Long, J. M., Powers, J., et al. (2014). Prevalence of mutations in a panel of breast cancer susceptibility genes in BRCA1/2-negative patients with early-onset breast cancer. *Genetics in Medicine, 11*(10), 176. doi:10.1038/gim.2014.

Oliver, A., & Greenberg, C.C. (2009). Measuring outcomes in oncology treatment: the importance of patient-centered outcomes. *Surg Clin North Am,* 89, 17-25.

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., et al. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus

recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*, 17: 405-424.

Spittler, C.A., Pallikathayil, L., & Bott, M. (2012). Exploration of how women make treatment decisions after breast cancer diagnosis. *Oncol Nurs Forum,* 39(5), E425-33.

Stacey, D., Legare, F., Col, N.F., Bennett, C.L., Barry, M.J., Eden, K.B., et al. (2014). Decision aids for people facing health treatment or screening decisions. *Cochrane Database Syst Rev,* 28(1), CD001431.

Vos, J., Otten, W., van Asperen, C., Jansen, A., Menko, F., & Tibben, A. (2008). The counsellees' view of an unclassified variant in BRCA1/2: recall, interpretation, and impact on life. *Psychooncology, 17*(8), 822–830.

Yu, J.H., Harrell, T.M., Jamal, S.M., Tabor, H.K., Bamshad, M.J. (2014). Attitudes of genetics professionals toward the return of incidental results from exome and whole genome sequencing. *Am J Hum Genet,* 95, 77-84

Zeelenberg, M., & Beattie, J. (1997). Consequences of regret aversion 2: additional evidence for effects of feedback on decision making. *Organization Behavior and Human Decision Processes*, 72, 63-78

Zeelenberg, M., & Pieters, R. (2007). A theory of regret regulation 1.0. *Journal of Consumer Psychology*, 17, 3-18

CHAPTER FIVE: MANUSCRIPT THREE

Disclosure of Genetic Variants of Uncertain Significance in an Exome Cohort

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Abstract

Purpose: This study described the effect of disclosing genetic variants of uncertain significance results on recipients' intentions to pursue health related behaviors.

Methods: We reported on 79 ClinSeq® study participants who received cardiomyopathy-associated genetic variants of uncertain significance results. With a conceptual framework adapted from the health belief model, a predictive correlational design was used to describe the impact of recipients' perceptions on intentions to pursue health related behaviors following disclosure of their results.

Results: Most participants (79%) intended to seek more information about their result in the future, over 80% intended to share their result with family members and health-care providers, and 46% intended to use result to change their lifestyle and health behaviors. Perceived risk, perceived severity, perceived value of information, self-efficacy, and sex explained 42.5% (Adjusted R^2 = 38.6%) of the variance in health behavior intentions of recipients of VUS results. Perceived value of information was the strongest predictor of health behavior intentions (β = 0.529, $p < 0.001$). Behavior intentions was moderately correlated with optimism ($r = 0.25$, $p = 0.03$), and weakly correlated with resilience ($r = 0.11$, $p = 0.614$) and tolerance of uncertainty ($r = -0.05$, $p = 0.669$).

Conclusions: Our findings suggest that perceived benefits associated with receipt of uncertain genetic information are more likely to motivate recipients to pursue health related behaviors than discussions of possible risk and severity associated with results.

Introduction

Developments in sequencing technologies are expected to enable a more personalized approach to patient care and disease risk stratification. Next generation sequencing can identify the genetic cause of a disease, but can also identify variants underlying diseases that are not being sought (secondary or incidental findings). This increase in genomic interrogation and enthusiasm for receiving genomic information has led to ongoing debate focusing on whether incidental findings should be returned to research participants (Wolf et al., 2012; Ferriere & Van Ness, 2012). Although a few are expected to be clinically significant, the risk consequences of a majority of identified secondary variants are often unknown and therefore designated as variants of uncertain significance (VUS). A VUS result gives no clear indication as to whether or not the patient is at higher risk for developing a disease, and further evidence are necessary to determine the significance (or not) of the variant in question.

The growing efforts to make health care more patient-centered is driving the need to help researchers and health care providers communicate the uncertainties of genetic results to participants amidst the limited understanding and fragmented body of knowledge available on VUS interpretations (Han, Klein, & Arora, 2011).

There are no generally recognized standard or commonly accepted guidelines for providing clinical interpretation and recommendations for those receiving VUS results (Vink, van Asperen, Devilee, Breuning, & Bakker, 2005). The public's interest to learn their sequencing information is increasing (Graves et al., 2014; McGuire & Lupinski, 2010), however, the potential benefits associated with ambiguous medical test results

such as VUS are poorly defined due to the uncertainty associated with their pathogenicity. There is limited information describing how recipients respond to actual VUS results disclosure, which makes it challenging to infer what sort of personal meaning individuals might attribute to learning such results.

Uncertainty surrounding health risks can affect disease perceptions and change how one interprets risk with regards to developing disease. Personality traits such as resilience, optimism, and tolerance of uncertainty influence perceptions of risk, disease severity, benefits of taking action, and self-efficacy. To build resilience, an individual must be exposed to adversity or a threat to physical or psychological health. Despite this adversity or threat, the individual adapts to minimize or avoid a risk (Luthar, Cicchetti, & Becker, 2000). Resilience is resistance, recovery, or rebound of mental and physical health after a challenge, which results from an individual's interaction with societal, community, family, physiological, and cellular factors across the life course (Szanton & Gill, 2010). Resilience may be influenced by genetics, exposure and experience with adversity, the desire to succeed, and social support (Luthar, Cicchetti, & Becker, 2000; Szanton & Gill, 2010; Dyer & McGuiness, 1996). The tendency to hold optimistic beliefs about the future has been associated with better health-related behaviors, leading to lower incidence of cardiovascular disease (Kubzansky et al., 2001), better prognosis following heart surgery (Scheier et al., 1999), and greater longevity (Giltay et al., 2006). Worry is concern about future events in which there is uncertainty about the outcome. Tolerance of uncertainty may be very important in understanding worry and may play a key role in the etiology and maintenance of worry. Tolerance of uncertainty is the

tendency of an individual to consider it acceptable that a negative event may occur (Dugas, Gosselin, & Ladouceur, 2001). These personality traits indirectly influence health-related behaviors by influencing an individual's perceptions. There is dearth of information on the impact of VUS disclosure on risk perception, perceived severity, and intentions to pursue health-related behavior. This phenomenon remains unexplored in genes associated with cardiomyopathy susceptibility.

This study was part of an ongoing genetics study (ClinSeq®) at the National Institutes of Health (NIH). ClinSeq® is a longitudinal study of > 1,000 individuals with a spectrum of atherosclerosis from unaffected to severe, that have been evaluated by exome or genome sequencing and have a choice about what types of information they want returned to them (Biesecker et al., 2009). The initial focus of the ClinSeq® study was on atherosclerotic heart disease but a majority are healthy volunteers. The ClinSeq® study provides a novel opportunity for baseline assessment of participant preferences to learn about their individual DNA results. Participants are informed of the types of results that can be generated, including limitations in interpreting data, and lack of reporting of non-pathogenic variants. Participants completed a baseline survey during the enrollment about their intentions to receive genetic results, and were asked their general preferences and reasons for receiving results. A prior hypothetical study to learn the relative perceived value of the different categories of genetic findings (gene variants that predispose to treatable disease, untreatable disease, no effect, and VUS) among participants reported that although there was significantly less interest in VUS, it was not to the degree anticipated. Participants' attitudes and social norms were

significantly correlated in all four categories and each was independently correlated with intentions to receive results (Facio et al., 2013). Additionally, information about risks to future generations was viewed by the participants to be as valuable as information about personal health risk that can be mitigated. That hypothetical study concluded that the participants' responses reflected their confidence in the usefulness of sequence information, even information that was currently not interpretable such as VUS. An exploratory study of 322 ClinSeq® participants identified enthusiasm for learning all types of results, with expectations to learn more about the genetic factors that contribute to their health risks (Facio et al., 2011). However, the underlying perceived value of learning of VUS results, perceived risk and severity attributed to uncertain DNA information, and what will be done if such information were received was not adequately investigated.

In an effort to address the issue of returning secondary variants to research participants, Ng and colleagues selected 22 arrhythmia and 41 cardiomyopathy-associated genes, and analyzed exome sequencing data from 870 ClinSeq® participants (Ng et al., 2013). Participants were not pre-selected for personal or family history of arrhythmia, cardiomyopathy, or sudden cardiac death. Pathogenicity classes were assigned according to criteria in Appendix G. In order to gain a clear insight into the impact of receiving uncertain genetic findings on perceptions of recipients, it is important to extend the findings of hypothetical studies to actual participants facing these choices. The present study returned some of the cardiomyopathy-associated VUS

identified by Ng and colleagues to study the impact of returning uncertain DNA results on health-related behaviors.

Using the Health Belief Model (HBM) as a framework, this study examined the impact of receiving cardiomyopathy-associated VUS on the recipient's: perceived severity and perceived susceptibility (risk perception) to cardiomyopathy; perceived benefits (value) attached to acting on the result by pursuing health-related behaviors, and; perceived self-efficacy (competence) to execute the desired health-related behaviors. The HBM proposes that perceived susceptibility (risk) to a condition and perceived severity (seriousness) associated with the condition makes up an individual's threat perception, and that threat perception motivates action. According to the HBM, a particular action will only be adopted if the perceived benefits outweigh its perceived barriers. In addition, perceived self-efficacy and cues to action are needed to trigger or stimulate health-related behaviors, while diverse demographic and socio-psychological factors indirectly influence behavior by modifying perceptions (Janz & Becker, 1984; Champion & Skinner, 2008). Health-related behavior is an action related to decreasing the risk of a certain disease outcome.

This study aimed to describe the impact of VUS genetic results on participants' intentions to pursue health-related behaviors. Levels of perceived risk, perceived severity, perceived value of information (benefit), and self-efficacy were used to predict recipients' intentions of pursuing health-related behaviors. The indirect influences of resilience, optimism, and tolerance of uncertainty as modifying factors on health-related behaviors were also examined.

Specific Aims

Aim 1: Describe the effect of perceived risk and perceived severity associated with disclosure of genetic variants of uncertain significance results on health behavior intentions.

Hypothesis $_1$: High levels of perceived risk and severity lead to high intentions to pursue health related behaviors.

Aim 2: Describe the effect of perceived value of information and self-efficacy associated with disclosure of genetic variants of uncertain significance results on health behavior intentions.

Hypothesis $_2$: High levels of perceived information value and self-efficacy increase intentions to pursue health related behaviors.

Aim 3: Examine the influence of resilience, optimism, and tolerance of uncertainty on health behavior intentions post disclosure of genetic variants of uncertain significance results.

Hypothesis $_3$: Resilience, optimism, and tolerance of uncertainty have moderating influence on intentions to pursue health related behaviors.

Methods

Study Design

A predictive correlational design was used for this study. Cardiomyopathy-associated VUS results were disclosed to participants to assess their post-disclosure levels of perceived risk and perceived severity, perceived value of information and self-efficacy. These factors were used to predict participants' intentions to pursue health-related behaviors associated with receipt of uncertain genetic information.

Sample

This correlational study was reviewed and approved by the NIH's National Human Genome Research Institute and the Johns Hopkins University Institutional Review Boards. Research participants were identified and recruited from the ClinSeq® project, a longitudinal genetics project that aims to apply large-scale medical sequencing in a clinical context to address the genetic basis of health, disease, and drug response. The selection criteria for participants and study design are published (Biesecker, Mullikin, Facio et al., 2009). Over 1000 Participants between 45 and 65 years old have been consented for initial phenotyping, exome and genome sequencing, and return of results. Participants with a range of coronary artery disease risk were enrolled and grouped into 4 bins based on their 10-year Framingham risk calculation (<5%, 5%-10%, >10%, known coronary artery disease). Participants were not selected for personal history of arrhythmia, cardiomyopathy or family history of sudden cardiac death (SCD). Informed consent at time of enrollment to ClinSeq® included description of exome and genome sequencing,

types of results that can be generated by sequencing technology, choices to receive results, limitations in interpreting data, lack of reporting of nonpathogenic variants.

Power Analysis

Using the HBM as their conceptual foundation, Shiloh and Saxe measured the relation of perceived risk and behavior intentions, and reported a correlation coefficient (r) of 0.41, and effect size (f^2) = 0.20 (Shiloh & Saxe, 1989). Also, a meta-analysis of studies examining the relationship between risk perception and health behavior related to vaccination reported a pooled r = 0.24, and effect size (f^2) = 0.08 (Brewer et al., 2007). Averaging these two correlation data, an r = 0.32 was used to estimate *a priori* sample sizes needed to detect a small to medium effect size (f^2 = 0.11) at a 0.05 significance level. A total sample size of 74 was estimated to detect the effect size of f^2 = 0.11 at a significance level of 0.05, with power = 0.80 (Table 5.6). To increase statistical power above 80%, a sample size of 81 was used for this study.

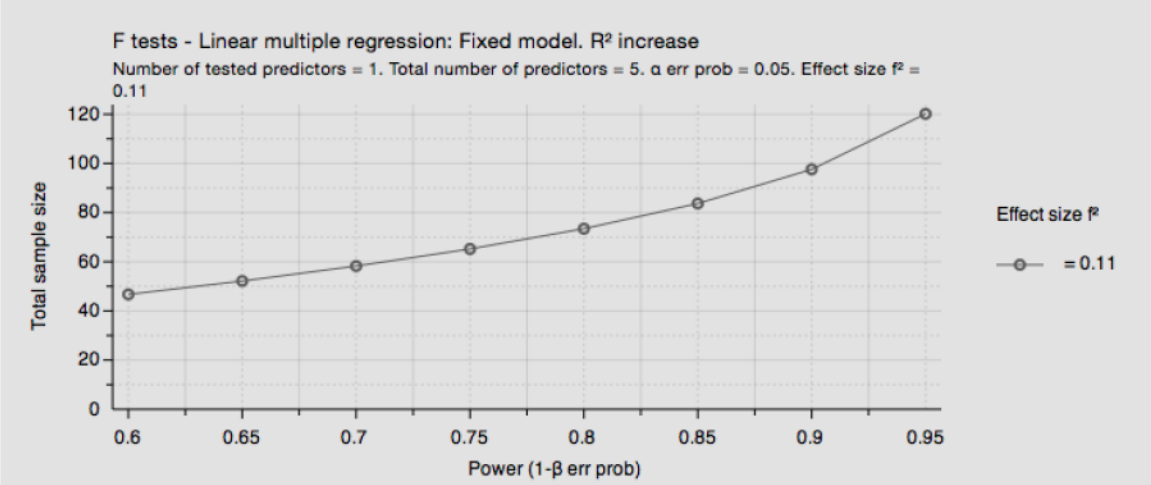


Figure 5.1: Power Analysis Calculation (G Power software – http://gpower.hhu.de/)

Procedures

One hundred and seventeen ClinSeq® participants with exome-generated variants of uncertain significance (VUS) in one of 20 cardiomyopathy-associated genes were eligible to participate in this study. Multiple attempts were made to contact eligible participants of which 81 were successfully consented and 36 were not consented for multiple reasons (requirement to travel to NIH for result -12; result too uncertain – 3; made initial contact but did not return calls or letters – 5; flagged by medical geneticist for cardiac related health concerns – 5; enrolled in on-going ClinSeq® study – 5; deceased – 2; lost to follow-up – 2; withdrawn from ClinSeq® - 2). The demographic characteristics of the study sample are outlined in Table 5.1

Medical and family histories of participants were reviewed with a medical geneticist (D.N.) familiar with the study. Upon obtaining verbal consent of participants' interest in learning results, VUS's were confirmed using Sanger sequencing in accordance with Clinical Laboratory Improvement Amendments (CLIA) validation regulations.

Participants gave written consents prior to disclosure of results. Average time of disclosure sessions was 50 minutes. Participants were surveyed 2 weeks post-VUS disclosure to assess perceived risk and perceived severity associated with cardiomyopathy, perceived information value of receiving VUS results, self-efficacy associated with capability to perform recommended actions, and intentions to pursue health-related behaviors.

Data Analysis

Descriptive statistics were used to summarize demographic information. Study variables were also examined by descriptive statistics using frequencies for ordinal/categorical variables and means and standard deviations for continuous variables. Bivariate analyses were performed to examine correlations of perceived risk, perceived severity, perceived information value, and self-efficacy to health behavior intentions. Collinearity diagnostics were performed to ascertain if multicollinearity was a valid concern. A standard measure of internal consistency (Cronbach's alpha) was calculated for the scales used in the study. A t-test was performed to investigate the effect of VUS sub-classification (VUS-High and Low) on health behavior intentions. Finally, the extent that health behavior intentions could be considered a function of perceived risk, perceived severity, perceived value of information, and self-efficacy was assessed through multiple linear regression analysis. Interactions of modifying factors such as resilience, optimism, and tolerance of uncertainty were also fitted to the model to capture their effects on health behavior intentions. A two-tailed p-value of <0.05 was considered statistically significant.

Results

Eighty percent of the participants in this study were white and had at least a college education. They ranged in age from 51 to 73 years, and 48% were female (Table 5.1). These demographic characteristics are consistent with those of larger samples of the ClinSeq® population (Lewis, Han, Hooker, et al., 2015). Forty-three percent were

told their results, albeit a VUS was less likely to have an effect on the gene's function (VUS-Low), while 57% were told the likelihood of their results affecting the gene's function was high (VUS-High) compared to other VUS results. Prior to enrollment, 19% of study participants had personal history of coronary artery disease (Bin 4).

Regarding the primary outcome of health behavior intentions, 79.5% intended to seek more information about their results in the future; 82.3% intended to share results with their healthcare provider; 46.8% intended to use information to change their lifestyle and health behavior, 30.4% did not intend to change, and 22.8% were unsure; and 81.8% intended to share results with family members, 11.7% were unsure.

Using a t-test, there was no difference in intentions between the two sub-classes of VUS results (High versus Low) (Table 5.2). A series of bivariate analyses was done to examine the correlation of predictor variables to health behavior intentions. As shown in Figure 5.2 and Table 5.3, the analysis revealed a correlation of health behavior intentions to perceived risk ($r = 0.18$, $p = 0.121$); perceived severity ($r = -0.21$, $p = 0.059$); perceived value of information ($r = 0.60$, $p < 0.001$); and self-efficacy ($r = 0.29$, $p = 0.009$). The variance inflation factor (VIF) from collinearity diagnostics was less than 10 for all four variables, and the mean VIF was 1.35. This showed that multicollinearity was not an issue. The covariate of sex showed a moderate correlation with health behavior intentions; with men displaying higher behavior intentions post VUS disclosure ($r = -0.22$, $p = 0.054$) compared to women. The covariate of sex slightly failed to reach statistical significance but was included in the final model to control for a potential confounding effect. Multiple linear regression analyses were conducted to determine

the level of variance in intentions explained by a model containing perceived risk, perceived severity, perceived value of information, self-efficacy, and sex. The model explained 42.5% (Adjusted R^2 = 38.6%) of the variance in health behavior intentions of recipients of VUS results, F (5, 73) = 10.79, p <0.001. Perceived risk, perceived severity, self-efficacy, and sex did not have statistically significant effects. Perceived information value was the strongest predictor of intentions in this model, with β = 0.530, p <0.001. For each standard deviation change in perceived information value, health behavior intention changed by over 0.5 standard deviation units (Table 5.4).

Each of the modifying factors (resilience, optimism, and tolerance of uncertainty) were dichotomized into high and low responses, and correlated to behavior intentions (Table 5.3). Optimism showed positive correlation to behavior intentions (r = 0.467, p = 0.025), while resilience and tolerance of uncertainty showed weak correlations (r = 0.111, p = 0.615; r = 0.312, p = 0.147) respectively. The calculated measures of internal consistency (Cronbach's alpha) for the scales used in this study are outlined in Table 5.5. Testing the interaction term of each modifying variable with each predictor variable in the regression model was not statistically significant. The slopes of the predictor variables were not different across both levels (high and low) of resilience, optimism, and tolerance of uncertainty. Therefore, no interaction terms were included in the final multiple regression model.

Table 5.1: Demographic Characteristics

Characteristic	Participant, n (%)
Race/Ethnicity	
White	65 (80)
Asian and not Hispanic or Latino	11 (14)
Hispanic or Latino	2 (2)
African-American	3 (4)
Education	
High school	2 (2)
Some college/Technical school	11 (14)
College	26 (32)
Postgraduate	39 (48)
Not reported	3 (4)
Age (years)	
50-65	51 (63)
66-75	30 (37)
Sex	
Female	39 (48)
VUS	
Low	35 (43)
High	46 (57)
Bins	
1-3	66 (81)
4	15 (19)

VUS = variant of uncertain significance, sub-classified into high versus low; Bins = based on 10-year Framingham risk calculation (1 = <5%, 2 = 5%-10%, 3 = >10%, 4 = known coronary artery disease).

Table 5.2: Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
1	34	4.658088	.1625188	.947639	4.327441	4.988735
2	45	4.653175	.1360332	.9125387	4.379018	4.927332
combined	79	4.655289	.1037085	.9217813	4.448822	4.861757
diff		.0049136	.2108124		-.4148675	.4246947

diff = mean(1) - mean(2) t = 0.0233

Ho: diff = 0 degrees of freedom = 77

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0

Pr(T < t) = 0.5093 Pr(|T| > |t|) = 0.9815 Pr(T > t) = 0.4907

Note: Group 1 = VUS-Low; Group 2 = VUS-High

Table 5.3: Correlation Analyses of Intentions and Predictor Variables

	Intentions	Perceived Risk	Perceived Severity	Perceived information Value	Perceived Self-efficacy	Sex
Intentions	1.0000					
Perceived Risk	0.1761 0.1206	1.0000				
Perceived Severity	-0.2135 0.0589	0.0589 0.6061	1.0000			
Perceived information Value	**0.6035*** **0.0000**	0.1447 0.2034	-0.1100 0.3345	1.0000		
Perceived Self-efficacy	**0.2905** **0.0094***	-0.1014 0.3739	**-0.3513*** **0.0015**	0.1676 0.1400	1.0000	
Sex	-0.2176 0.0541	-0.1024 0.3691	-0.1539 0.1756	**-0.3106*** **0.0053**	0.0689 0.5465	1.0000

* Statistically significant at the 0.05 level

Correlation Analyses of Intentions and Modifying Factors

	Intentions	Resilience	Optimism	Tolerance of Uncertainty
Intentions	1.0000			
Resilience	0.1108 0.6147	1.0000		
Optimism	0.0718 0.7447	**0.5165*** **0.0116**	1.0000	
Tolerance of Uncertainty	0.3123 0.1468	-0.2241 0.3040	-0.2260 0.2998	1.0000

* Statistical significance at 0.05 level

Table 5.4: Multiple Regression Analyses for Predicting Health Behavior Intentions (n=79)

Predictor Variable	Coefficient	B	Std. Err.	t	p-value
Perceived Risk	.1217759	.118306	.0932895	1.31	0.196
Perceived Severity	-.1571783	-.1089677	.1400604	-1.12	0.265
Perceived Information Value	.6992928	.5299557	.1269975	5.51	**0.000 ***
Self-efficacy	.1824311	.1731582	.1017038	1.79	0.077
Sex	-.091303	-.0498058	.1752817	-0.52	0.604

* Statistically significant at the 0.05 level

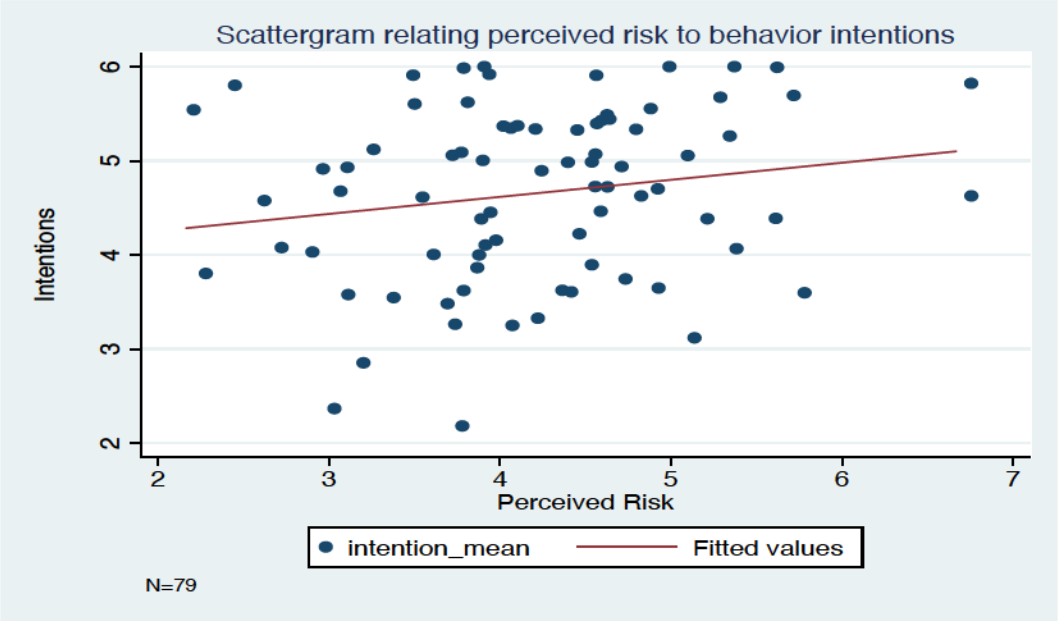
Table 5.5: Instruments Measure of Internal Consistency

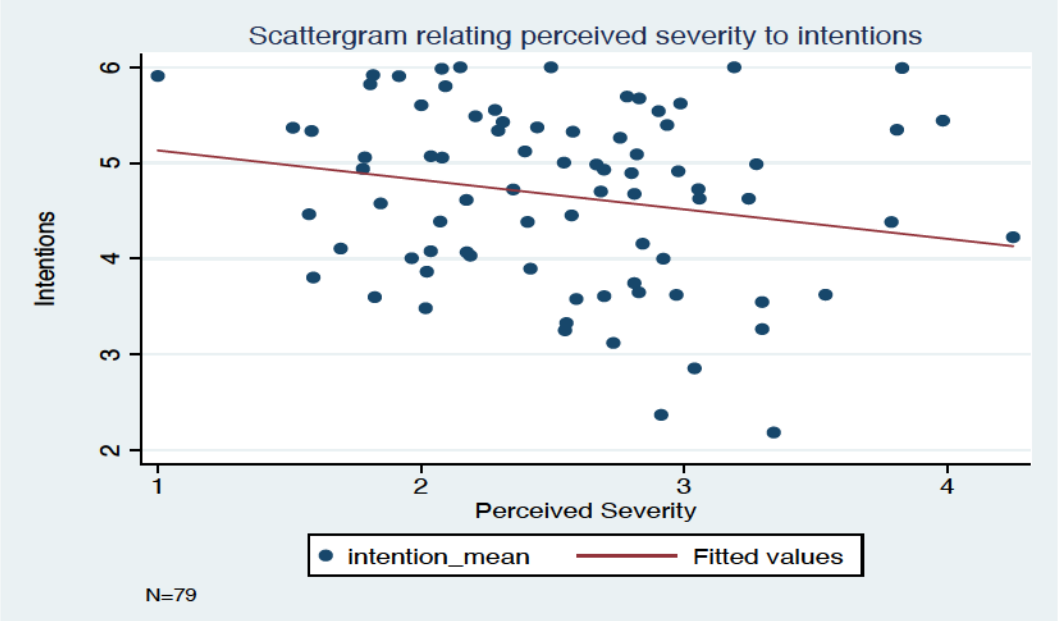
Instrument	**Cronbach's alpha**
Perceived Risk	0.84
Perceived Severity	0.78
Perceived Information Value	0.87
Self-efficacy	0.89
Intentions	0.83

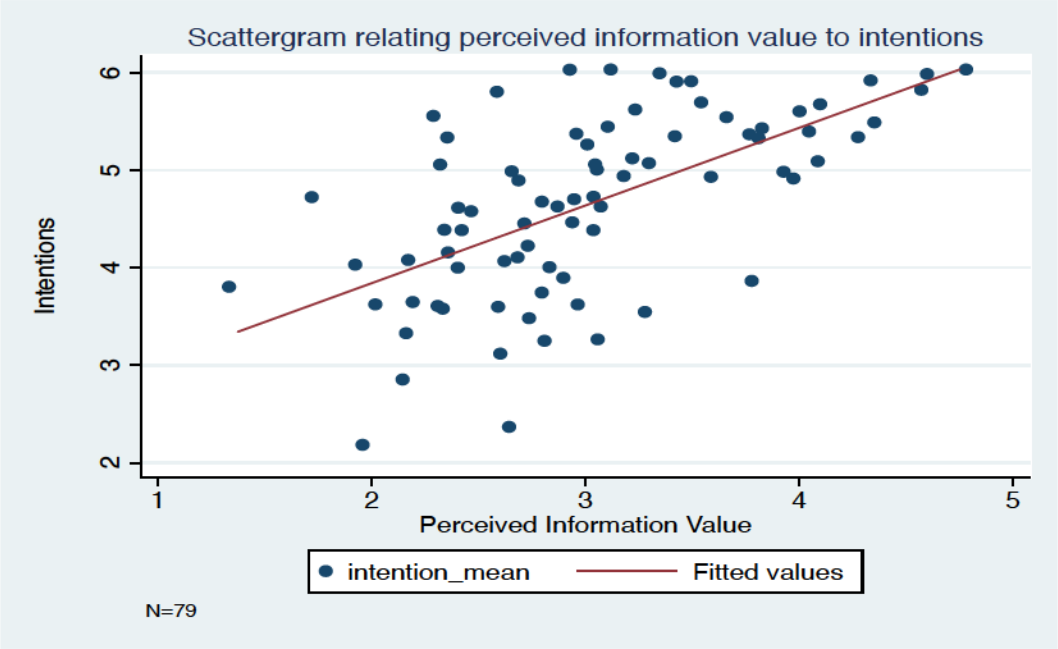
Table 5.6: Power Analysis

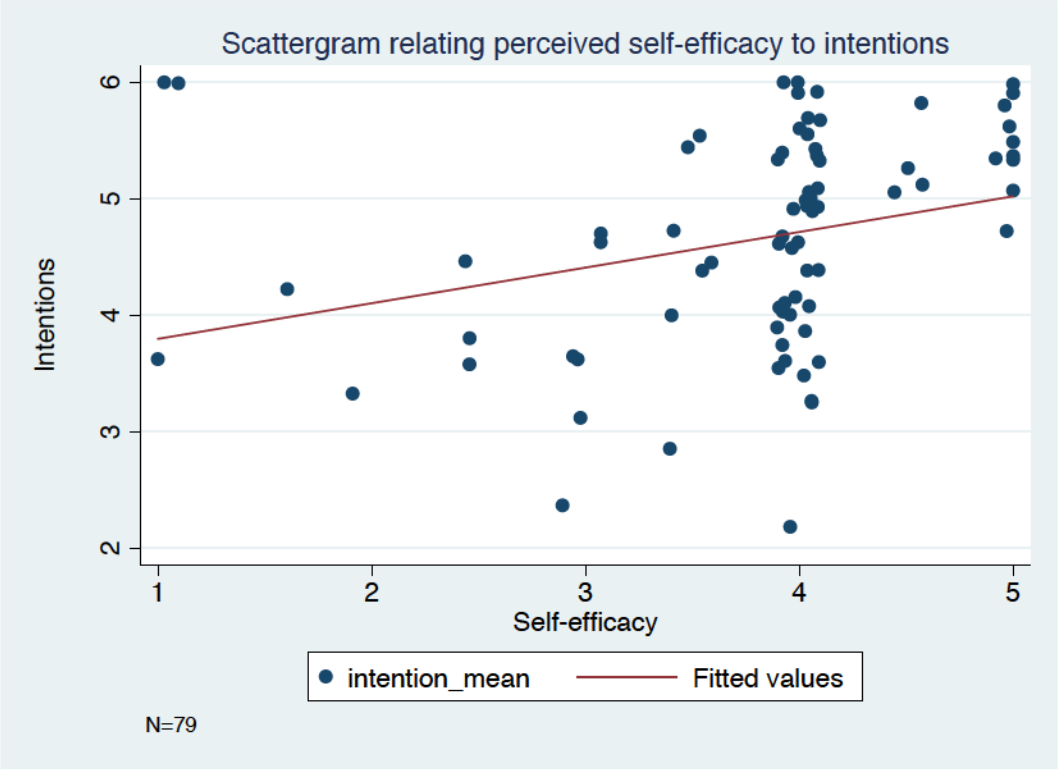
Power (1-β)	**Total Sample Size**
0.60	47
0.65	52
0.70	58
0.75	65
0.80	**74**
0.85	84
0.90	98
0.95	120

Figure 5.2: Scattergrams of Correlation Analyses of Intentions and Predictor Variables.






Scattergram relating perceived information value to intentions
Intentions
2
3
4
5
6
1
2
3
4
5
Perceived Information Value
intention_mean
Fitted values
N=79


Scattergram relating perceived self-efficacy to intentions
Intentions
2
3
4
5
6
1
2
3
4
5
Self-efficacy
intention_mean
Fitted values
N=79

Discussion

The findings in this study describe the influence of perceptions on intentions to pursue health related behaviors following the receipt of uncertain genetic information. Intention is the most proximal predictor of behavior (Azjen, 1991). In this sample of well educated, post reproductive research participants, perceptions that predict behavior such as perceived risk, perceived severity, perceived information value, and perceived self-efficacy were assessed following disclosure of cardiomyopathy-associated genetic VUS to consented recipients.

Among the factors predicted to affect behavior by the HBM (Rosenstock, Strecher, & Becker, 1988), perceived benefit, operationalized as perceived value of information emerged as the strongest predictor of behavior intentions in this cohort of VUS recipients ($\beta = 0.530$, $p < 0.001$). Perceived risk, perceived severity, and self-efficacy had weak statistically insignificant effects in predicting behavior intentions, all except perceived severity were in the directions predicted by the HBM.

The HBM argues that individuals will be motivated to change behavior if perceptions of severity and risk (susceptibility) to negative health outcomes are strong. Risk and severity relate to the individual's perceptions of the negative health outcome, while perceived benefit and self-efficacy concern the individual's perception of the behavior that will reduce the likelihood of the negative outcome. If the undesirable health outcome will not have a large effect on a person's life, he or she will not be motivated to act to avoid it (Rosenstock, Strecher, & Becker, 1988).

Similar to our findings, a meta-analysis of 18 studies conducted to determine whether the dimensions of the HBM could longitudinally predict behavior showed that perceived benefits are stronger predictors of behavior compared with the inconsistent effects and weakness of perceived risk and severity (Carpenter, 2010). Participants ascribed benefits to learning their VUS results even after they were told the uncertain information did not have clinical utility due to lack of genetic evidence. There are 4 plausible explanations for this finding. First, the element of personal utility ascribed to disclosed information by ClinSeq® participants range from satisfying a curiosity and peace of mind, to personal control and increased self-awareness, to motivation to pursue positive health behaviors in general (Lewis et al., 2015). Over 80 percent of VUS recipients in this study intend to share results with family members and their healthcare providers. This is consistent with stated intentions of ClinSeq® participants to use results to improve their health and those of family members (Facio et al., 2011; Wright et al., 2014) and findings from other hypothetical studies (Hendersen et al., 2008). Second, our participants' understanding of uncertainty surrounding genome sequencing may have contributed to the perceived value associated with VUS results. Individuals that perceived uncertainty in genome information as normal or expected viewed uncertain information more positively and even valuable as an opportunity, compared with individuals not expecting uncertainty and perceiving it as a threat (Biesecker et al., 2014). Third, some participants have expressed altruistic reasons for enrolling in the study. Independent of any personal benefit, some participants believe that their information would contribute to scientific progress and benefit others (Wright et al.,

2014). Finally, recipients of VUS results were informed of the potential for reclassification as additional genetic evidence become available. Suggestions that the understanding of uninterpretable genetic information may evolve could have positively influenced their perceptions.

Forty-seven percent of participants intended to use the disclosed information to change their lifestyle and health-related behaviors. This could indicate that over half of participants may already be involved in health-promoting behaviors and lifestyles activities to reduce potential cardiac disease risks.

The weak effects of perceived risk and severity on predicting health behavior intentions suggest that participants learning their cardiomyopathy-associated VUS results may not perceive themselves at risk for developing the condition, and may have determined that cardiomyopathy is not a serious condition that could affect their lives. These calculations could be based on negative personal and family histories of cardiomyopathy, review of previous heart diagnostics (echocardiogram and electrocardiogram) during return of results sessions, or false reassurance that a more significant (pathogenic or likely pathogenic) finding would have been detected from analyzing their exome sequence. Overall, our findings are consistent with other studies showing low estimates in the relationships between perceived risk and severity on the likelihood of adopting a behavior (Harrison, Mullen, & Green, 1992; Carpenter, 2010). The negative correlation of perceived severity with behavior intentions was unexpected. The information provided on diagnosis and management of cardiomyopathy during our disclosure sessions may have influenced perceptions related to the seriousness of the

condition. Participants were told that the life expectancy of individuals diagnosed with cardiomyopathy is close to normal with appropriate treatment and management of the condition (Sen-Chowdhry et al., 2016).

The impact of perceived self-efficacy has been well described in the paradigm of health behavior (Bandura, 1977). High levels of self-efficacy are associated with increased willingness to pursue health related behaviors (Kamimura et al., 2016; Bartfield et al., 2010). Although the positive correlation of behavior intentions and self-efficacy in this study was statistically significant ($r = 0.29$, $p = 0.009$), the contribution of self-efficacy to predicting health behavior intentions was weak ($\beta = 0.173$, $p = 0.077$). A previous study reported that response efficacy, mediated by attitudes towards receiving results, explained a significant amount of the variance in intentions (Wade et al., 2011) but did not explore intentions to act on the testing results.

Although not statistically significant, male participants in this study reported higher health behavior intentions than female participants ($p = 0.054$). The prevalence of coronary heart disease in men over 40 years of age is higher than in women of similar age (Mozaffarian et al., 2015). Therefore, men in this study are more likely to have experienced heart disease compared to women. This may partly explain the reported increase in behavior intentions in men. The behavior of men and women is influenced by several unstable and fluctuating factors, such as the degree to which it is perceived as socially desirable, degree to which it is held with high certainty, and the degree to which it is clearly conveyed by situational cues (Deaux & Major, 1987). In general, the more socially desirable or positive individuals perceive the expected behavior as being,

the more likely they are to provide confirming evidence (Shrauger, 1982). Men and women may differ in their interpretation of the desirability of a particular behavior. Although the opposite was noted in this study, women are generally higher in social desirability (tendency to respond in such a way as to avoid criticism) than men (Herbert et al., 1995). Social desirability and approval have been shown to produce biases in self-reported surveys (Kelly, Soler-Hampejsek, Mensch, & Hewett, 2013; Catania, Gibson, Chitwood, & Coates, 1990; Larsen, Martin, Ettinger, & Nelson, 1976) and may partly explain the gender-related differences in behavior intentions in this study. Biases due to social desirability or social approval can confound the relation between variables; therefore the covariate of sex was included in the final model.

Behavior intentions was moderately correlated with tolerance of uncertainty (r= 0.31, p = 0.147), and weakly correlated with resilience (r=0.11, p = 0.615) and optimism (r= 0.07, p = 0.745). Resilience and optimism are negatively associated with perceived uncertainty (Madeo, O'Brien, Bernhardt, & Biesecker, 2012; Macnamara, dissertation, 2014) and perceived risk (Persoskie, Ferrer, Nelson, & Klein, 2014). The positive relationships among behavior, resilience and optimism have also been described (Youssef, 2007). The tendency to hold optimistic beliefs about the future has been associated with better engagement in health-related behaviors (Scheier et al., 1999; Giltay, Kamphuis, Kalmijin, Zitman, & Kromhout, 2006). The findings that resilience, optimism, and tolerance of uncertainty did not modify relationships between perceptions and behavior intentions was not anticipated, but could be related to certain characteristics of our study participants. As a cohort, they have demonstrated

enthusiasm for genome sequencing technology, displaying high levels of resilience, optimism, and tolerance of uncertainty, and represent a segment of the public that are likely to pursue genetic technology for reasons beyond health concerns (Facio et al., 2011). Further work is needed to examine this hypothesis using a cohort with different characteristics.

Informing participants of the sub-classification of their VUS results into a high and low group did not reveal differences in behavior intentions. This finding is suggestive that research participants respond to the classification of results as uncertain, and efforts to further divide this class of variant may not have measurable utility to recipients. The five-category classification system recommended by the American College of Medical Genetics and Genomics (ACMG) (Richards et al., 2015) does not address the conundrum of having a wide range of posterior probabilities (0.10 – 0.90) for VUS variants, which makes it challenging to provide useful concrete clinical recommendations. Sub-classification of the VUS results was an attempt to examine recipients' perceptions and response to information that were either closer to the lower range (VUS-Low) or the upper range (VUS-High) of VUS posterior probabilities. The classification of VUS into higher or lower-risk categories will remain an important exercise in clinical genetics as an increasing number of VUS are identified using next generation sequencing. Currently, many groups and laboratories are cautious in classifying variants as pathogenic unless there is strong evidence of association with disease, therefore most variants are listed as VUS's (Plon et al., 2008). As more genetic

testing for hereditary diseases move into clinical practice, the problem associated with their interpretation will present challenges to health care providers and patients.

The findings of this study provide an understanding of how perceptions related to uncertain genetic information informs health related behaviors. Limitations to this study are the descriptive nature of this research among a relatively small number of participants that are predominantly white, highly educated, and older than reproductive age, and as such may not be representative of the general public. A replication of this study in a cohort with distinctly different sociodemographic attributes will contribute to our understanding of perceptions associated with disclosure of uncertain information from genome sequencing. While we noted no difference in behavior intentions between the two VUS sub-groups, the study was not experimental, and therefore not powered to test for a difference. Future work to examine the impact of VUS results sub-classification is needed to determine whether it influences recipients' responses, and worth the research effort it would take to generate. Finally, these participants self-selected to participate in a genetics study and therefore could be more motivated to learn and act on their results than other groups of individuals.

Conclusions

Uncertainty surrounding health risks can affect disease perceptions and change how one interprets risk with regards to developing disease. Our findings indicate that perceived value of information is a strong positive predictor of participants' intentions to pursue health related behaviors following disclosure of uncertain genetic

information. Participants' perceived benefit from learning their cardiomyopathy-associated VUS results was evidenced by high intentions to communicate results to family members and health-care providers. Perceptions of risk and severity of cardiomyopathy, and self-efficacy did not have significant impacts on intentions to pursue health related behaviors. To adequately manage participant expectations regarding the utility of uncertain genomic information, more work is needed to examine the effects of personality traits such as resilience, optimism, and tolerance of uncertainty on the behavior of research participants with high enthusiasm for learning genetic information of any significance. Our findings suggest that perceived benefits associated with receipt of uncertain genetic information is more likely to motivate recipients to act than discussions of possible risk and severity associated with their results.

References

Azjen, I. (1991). The theory of planned behavior. *Org Behav & Hum Decis Proces,* 50, 179-211.

Bandura, A. (1977). Self-efficacy: towards a unifying theory of behavior change. *Psychol Rev,* 84, 191-215.

Bartfield, J.K., Ojehomon, N., Huskey, K.W., Davis, R.B., & Wee, C.C. (2010). Preferences and self-efficacy for diet modification among primary care patients. *Obesity,* 18(2), 430-432.

Biesecker, B.B., Klein, W., Lewis, K.L., Fisher, T.C., Wright, M.F., Biesecker, L.G., et al. (2014). How do research participants perceive "uncertainty" in genome sequencing? *Genet Med,* 16(12), 977-980.

Biesecker, L.G., Mullikin, J.C., Facio, F.M., Turner, C., Cherukuri, P.F., Blakesley, R.W., et al. (2009). The ClinSeq Project: piloting large-scale genome sequencing for research in genomic medicine. *Genome Res,* 19, 1665-1674.

Brewer, N.T., Chapman, G.B., Gibbons, F.X., Gerrard, M., McCaul, K.D. & Weinstein, N.D. (2007). Meta-analysis of the relationship between risk perception and health behavior: the example of vaccination. *Health Psychol,* 26(2), 136-145.

Carpenter, C.J. (2010). A meta-analysis of the effectiveness of health belief model variables in predicting behavior. *Health Commun,* 25(8), 661-669

Catania, J.A., Gibson, D.R., Chitwood, D.D., & Coates, T.J. (1990). Methodological problems in AIDS behavioral research: influences on measurement errors and participation bias in studies of sexual behavior. *Psychol Bull,* 108(3), 339-362.

Champion, V.L., & Skinner, C.S. (2008). The health belief model. Glanz K, Rimer BK, Viswanath K (Eds.). *Health behavior and health education: theory, research, and practice*. San Francisco, CA: Jossey-Bass.

Deaux, K., & Major, B. (1987). Putting gender into context: an interactive model of gender-related behavior. *Psychological Review,* 94(3), 369-389.

Dugas, M.J., Gosselin, P., & Ladouceur, R. (2001). Intolerance of uncertainty and worry: investigating narrow specificity in a non-clinical sample. *Cognitive Therapy and Research,* 25, 551-558.

Dyer, J.G., & McGuiness, T.M. (1996). Resilience: Analysis of the concept. *Archives of Psychiatric Nursing,* 10, 276-282.

Facio, F.M., Brooks, S., Loewenstein, J., Green, S., Biesecker, L.G., & Biesecker, B.B. (2011). Motivators for participation in a whole-genome sequencing study: implications for translational genomics research. *Eur J Hum Genet,* 19, 1213-1217.

Facio, F.M., Eidem, H., Fisher, T.,Brooks, S., Linn, A., Kaphingst, K.A., et al. (2013). Intentions to receive individual results from whole-genome sequencing among participants in the ClinSeq study. *Eur J Hum Genet*, 21(3), 261-265.

Ferriere, M., & Van Ness, B. (2012). Return of individual research results and incidental findings in the clinical trials cooperative group setting. *Genet Med,* 14, 411-416.

Giltay, E.J., Kamphuis, M.H., Kalmijin, S., Zitman, F.G., & Kromhout, D. (2006). Dispositional optimism and the risk of cardiovascular death: The Zutphen Elderly Study. *Archives of Internal Medicine*, 166, 431–436.

Graves, K.D., Sinicrope, P.S., Esplen, M.J., Peterson, S.K., Patten, C.A., Lowery, J., et al. (2014). Communication of genetic test results to family and health-care providers following disclosure of research results. *Genet Med,* 16(4), 294-301.

Han, P.K., Klein, W.M., & Arora, N.K. (2011). Varieties of uncertainty in health care: a conceptual taxonomy. *Med Decis Making*, 31(6), 828-838.

Han, P.K., Klein, W.M., Lehman, T., Killam, B., Massett, H., & Freedman, A.N. (2011). Communication of uncertainty regarding individualized cancer risk estimates: effects and influence factors. *Med Decis Making,* 31(2), 354-366.

Harrison, J.A., Mullen, P.D. & Green, L.W. (1992). A meta-analysis of studies of the health belief model with adults. *Health Education Research*, 7, 107–116.

Henderson, G., Garrett, J., Bussey-Jones, J., Moloney, M.E., Blumenthal, C., & Corbie-Smith, G. (2008). Great expectations: views of genetic research participants regarding current and future genetic studies. *Genet Med,* 10, 193-200.

Herbert, J.R., Clemow, L., Pbert, L., Ockene, I.S., & Ockene, J.K. (1995). Social desirability bias in dietary self-report may compromise the validity of dietary intake measures. *Int J Epidemiol*, 24, 389-398.

Janz, N.K., & Becker, M.H. (1984). The health belief model: A decade later. *Health Education & Behavior*, 11(1), 1-47.

Kamimura, A., Nourian, M.M., Jess, A., Chernenko, A., Assasnik, N., & Ashby, J. (2016). Perceived benefits and barriers and self-efficacy affecting the attendance of health education programs among uninsured primary care patients. *Eval Program Plann,* 59, 55-61.

Kelly, C.A., Soler-Hampejsek, E., Mensch, B.S., & Hewett, P.C. (2013). Social desirability bias in sexual behavior reporting: evidence from an interview mode experiment in rural Malawi. *Int Perspect Sex Reprod Health,* 39(1), 14-21.

Kubzansky, L.D., Sparrow, D., Vokonas, P., & Kawachi, I. (2001). Is the glass half empty or half full? A prospective study of optimism and coronary heart disease in the Normative Aging Study. *Psycho-somatic Medicine*, 63, 910–916.

Larsen, K.S., Martin, H.J., Ettinger, R.H., & Nelson, J. (1976). Approval seeking, social cost, and aggression: a scale and some dynamics. *J Psychol,* 94, 3-11.

Lewis, K.L., Han, P.K., Hooker, G.W., Klein, W.M., Biesecker, L.G., & Biesecker, B.B. (2015). Characterizing participants in the ClinSeq genome sequencing cohort as early adopters of a new health technology. *PLoS One*, 10:e0132690

Lewis, K.L., Hooker, G.W., Connors, P.D., Hyams, T.C., Wright, M.F., Caldwell, S., et al. (2015). Participant use and communication of findings from exome sequencing: a mixed methods study. *Genet Med,* 18(6), 577-583.

Luthar, S.S., Cicchetti, D., & Becker, B. (2000). The construct of resilience: A critical evaluation and guidelines for future work. *Child Development,* 71, 543-562.

Macnamara, E. (2014). The role of uncertainty in coping efficacy: the experience of parents of children with undiagnosed medical conditions. Dissertation thesis.

Madeo, A.C., O'Brien, K.E., Bernhardt, B.A., & Biesecker, B.B. (2012). Factors associated with perceived uncertainty among parents of children with undiagnosed medical conditions. *Am J Med Genet A,* 158A (8), 1877-1884.

McGuire, A.L., & Lupinski, J.R. (2010). Personal genome research: what should the participant be told? *Trends in Genetics, 26*(5), 199-201.

Mozaffarian, D., Benjamin, E.J., Go, A.S., et al. (2015). Heart disease and stroke statistics – 2015 update. *Circulation,* 131(4), e29-e322.

Ng, D., Johnston, J.J., Teer, J.K., Singh, L.N., Peller, L.C., Wynter, J.S., et al. (2013). Interpreting secondary cardiac disease variants in an exome cohort. *Circulation,* 6(4), 337-346.

Ouellette, J.A., & Wood, W. (1998). Habit and intention in everyday life: the multiple processes by which past behavior predicts future behavior. *Psychological Bulletin,* 124(1), 54-74.

Persoskie, A., Ferrer, R.A., Nelson, W.L., & Klein, W.M. (2014). Precancer risk perceptions predict postcancer subjective well-being. *Health Psychology,* 33(9), 1023-1032.

Plon, S.E., Eccles, D.M., Easton, D., Foulkes, W.D., Genuardi, M., Greenblatt, M.S., et al. (2008). Sequence variant classification and reporting: Recommendations for improving the interpretation of cancer susceptibility genetic test results. *Human Mutation*, 29(11), 1282-1291.

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., et al. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med,* 17(5), 405-424.

Rosenstock, I. M., Strecher, V. J., & Becker, M. H. (1988). Social learning theory and the health belief model. *Health Education Quarterly*, *15*, 175–183.

Szanton, S.L., & Gill, J.M. (2010). Facilitating resilience using a society-to-cells framework: A theory of nursing essentials applied to research and practice. *Advances in Nursing Science,* 33(4), 329-343.

Scheier, M.F., Matthews, K.A., Owens, J.F., Schulz, R., Bridges, M.W., Magovern, G.J., et al. (1999). Optimism and rehospitalization after coronary artery bypass graft surgery. *Archives of Internal Medicine*, 159: 829–835.

Schrauger, J.S. (1982). Selection and processing of self-evaluative information: experimental evidence and clinical implications. In G. Weary & H.L. Mirels (Eds.), *Integrations of clinical and social psychology* (pp.128-153). New York: Oxford University Press.

Sen-Chowdhry, S., Jacoby, D., Moon, J.C., & McKenna, W.J. (2016). Update on hypertrophic cardiomyopathy and a guide to the guidelines. *Nat Rev Cardiol*, 13(11), 651-675.

Shiloh, S., & Saxe, L. (1989). Perception of risk in genetic counseling. *Psychol & Health,* 3,

45-61.

Vink, G.R., van Asperen, C.J., Devilee, P., Breuning, M.H., & Bakker, E. (2005). Unclassified variants in disease-causing genes: nonuniformity of genetic testing and counselling, a proposal for guidelines. *EJHG*, *13*(5), 525-527.

Wade, C.H., Shiloh, S., Woolford, S.W., Roberts, J.S., Alford, S.H., Marteau, T.M., & Biesecker, B.B. (2012). Modelling decisions to undergo genetic testing for susceptibility to common health conditions: an ancillary study of the Multiplex Initiative. *Psychol Health,* 27(4), 430-444.

Wolf, S.M., Crock, B.N., Van Ness, B., Lawrenz, F., Kahn, J.P., Beskow, L.M., et al. (2012). Managing incidental findings and research results in genomic research involving biobanks and archived data sets. *Genet Med,* 14, 361-384.

Wright, M.F., Lewis, K.L., Fisher, T.C., Hooker, G.W., Emanuel, T.E., Biesecker, L.G., et al. (2014). Preferences for results delivery from exome sequencing/genome sequencing. *Genet Med,* 16, 442-447.

Youssef , C.M. (2007). Positive organizational behavior in the workplace: the impact of hope, optimism, and resilience. *Journal of Management,* 33(5), 774-800.

CHAPTER SIX: DISCUSSION

Introduction

The emergence of next generation sequencing as a clinical tool in aiding diagnosis is fueling enthusiasm to provide genomic information to patients (Arar et al., 2010; Bollinger et al., 2012). One consequence of this technology is that genetic results are more likely to include variants of uncertain significance (VUS). VUS are alterations in the DNA sequence of a gene that have an intermediate probability of pathogenicity or disease risk (Lindor et al., 2013). There are no universally accepted standards for reporting VUS results (Lindor et al., 2012). Also, conveying the potential implications of uncertain results to patients and research participants sufficiently to inform their decision-making is challenging.

Uncertainty can have a variety of psychological effects. Perceptions related to uncertainty are likely to predict decisions to learn genetic results and to act on the information (Biesecker et al., 2014). There is a need to examine the impact of the inherent uncertainty in genetic sequencing information on the perceptions of recipients. To this end, the goal of this study was to describe the effects of disclosing uncertain genetic information on intentions to pursue health related behaviors within the context of the Health Belief Model (HBM). To achieve that goal, the following four specific aims guided this dissertation study:

Aim 1: Describe the effects of perceived risk and perceived severity associated with disclosure of genetic variants of uncertain significance results on health behavior intentions.

Hypothesis $_1$: High levels of perceived risk and severity lead to high intentions to pursue health related behaviors.

Aim 2: Describe the effects of perceived value of information and self-efficacy associated with disclosure of genetic variants of uncertain significance results on health behavior intentions.

Hypothesis $_2$: High levels of perceived information value and self-efficacy increase intentions to pursue health related behaviors.

Aim 3: Examine the influence of resilience, optimism, and tolerance of uncertainty on health behavior intentions post disclosure of genetic variants of uncertain significance results.

Hypothesis $_3$: Resilience, optimism, and tolerance of uncertainty have moderating influence on intentions to pursue health related behaviors.

Aim 4: Measure the level of regret associated with decision to learn genetic variants of uncertain significance results 2 weeks post disclosure.

Hypothesis $_4$: The uncertainty associated with genetic VUS information increases regret with decision to learn result.

This chapter synthesizes the results, which are presented within the framework of the four study aims. Findings not presented in the previous chapters are highlighted here. Then, a discussion of the strengths and limitations of this dissertation research and the implications of the findings to health care practice and research are outlined. Finally, recommendations for further research are offered.

Summary of Findings by Specific Aim

Aim1: Influence of perceived risk and perceived severity on health behavior intentions post-VUS result disclosure

The perceived risk and perceived severity of a health threat describe an individual's perceptions of a negative health outcome. Although perceived risk and perceived severity are central to many health behavior theories (Brewer et al., 2007; Weinstein et al., 2007), our findings of no statistically significant relations with health-related behaviors have been reported in other studies. Janz and Becker (1984) speculated that the poor predictive effects of risk and severity perceptions might be due in part to the difficulty individuals have in conceptualizing these dimensions of the health belief model. Perceptions of risk or severity of a condition such as cardiomyopathy may be challenging especially when a respondent is asymptomatic or has had little or no personal experience. In individuals diagnosed as ill or experiencing

symptoms of a condition, the perceived severity dimension is more meaningful and takes on greater importance in predicting behavior than in otherwise healthy individuals. Carpenter (2010) reported perceived risk and severity as weak behavior predictors, with the effect of perceived risk almost near zero.

Plausible explanations for these findings are that our study participants, mostly self-referred to the parent longitudinal genetics study that started out with a focus on heart related conditions, may not vary in their perceived levels of risk or severity associated with heart diseases in general. Therefore, return of a cardiomyopathy-associated VUS could be perceived as confirmation for enrollment in the ClinSeq® study. The low estimates of the relationships between health behavior intentions and perceived risk ($r = 0.18$, $p = 0.121$), and perceived severity ($r = -0.21$, $p = 0.059$) in this study is similar to estimates reported in other studies (Harrison et al., 1992; Carpenter, 2010). If an undesirable health outcome will not have a large effect on a person's life, he or she will not be motivated to act to avoid it (Rosenstock, Strecher, & Becker, 1988). The negative correlation of perceived severity with behavior intentions was unexpected. The information provided to participants on the diagnosis and management of cardiomyopathy during our disclosure sessions may have influenced perceptions related to the seriousness of the condition. Although a rare symptom associated with cardiomyopathy is sudden death, participants were told that current screening guidelines have the potential to identify affected individuals at very early stages, leading to effective prevention of related complications (Quarta et al., 2016), and the life

expectancy of individuals diagnosed with cardiomyopathy is close to normal with appropriate treatment and management of the condition (Sen-Chowdhry et al., 2016).

Aim2: Influence of perceived value of information and perceived severity on health behavior intentions post VUS disclosure.

The perceived value of information was strongly correlated ($r = 0.60$, $p < 0.001$), and self-efficacy was weakly correlated ($r = 0.29$, $p = 0.009$) with health behavior intentions in this study. The perceived value of information was the strongest predictor of health behavior intentions in our model ($\beta = 0.530$, $p < 0.001$). This is consistent with previously reported findings in this cohort of participants (Wright et al., 2014) and other studies (Harrison et al., 1992; Harris et al., 2012). Hypothetical studies of ClinSeq® participants have reported perceived benefits as reasons for wanting their medically non-actionable and uncertain results. These perceived benefits range from satisfaction of curiosity, peace of mind, and control of personal information, to utilizing information to improve personal health and those of family members (Facio et al., 2013; Wright et al., 2014). The effect size of perceived benefit on behavior is stronger when the outcome was preventing a negative health outcome rather than treating an existing one (Carpenter, 2010).

Our participants' understanding of uncertainty surrounding genome sequencing may have contributed to the perceived value associated with VUS results. Individuals with perceptions of uncertainty in genome information as normal or expected view uncertain information more positively. Participants were asked on the survey to

describe in their own words what they understood a VUS to mean. Overall, a majority of participants were able to describe VUS results as genetic information with incomplete interpretations that may or may not have implications for their health. One participant said "I have a variation in a gene for which some mutations and variations are pathogenic, but mine has not been linked to any known diseases." Another participant noted, "It is not known at this particular time whether this finding in genetic change will have any implications for my future health."

The effect of self-efficacy in predicting health behavior intentions in this study was weak ($\beta = 0.173$, $p=0.077$), with higher self-efficacy leading to higher intentions to pursue health behavior. Self-efficacy explains a significant amount of the variance in behavior (Wade et al., 2011; Grembowski et al., 1993). Self-efficacy relates to beliefs about capabilities of performing specific behaviors in situations. The perceptions, and not the actual capabilities, is what influence behavior (Strecher et al., 1986). An individual's efficacy expectation will vary depending on the task and context to be addressed. To assess self-efficacy in this study, participants were asked whether they felt confident and competent in their ability to pursue health behaviors and use medical resources to manage their cardiomyopathy VUS results.

Over 82% of participants reported high self-efficacy in their ability to pursue behaviors related to their VUS results. High levels of perceived self-efficacy can have beneficial consequences for individual functioning and well-being such as initiation of preventive care or seeking early treatment, but also, individuals with high self-efficacy are more likely to rate their health as better compared to those with low perceived self-

efficacy (Bandura, 1977). The hypothesis of high levels of self-efficacy predicting health behavior intentions was not supported by our findings. It has been suggested that self-efficacy is a consequence and not a cause of behavior (Hawkins, 1992). Hawkins notes that self-efficacy is determined by prior events in a person's life and therefore not a logical predictor of behavior. This may partly explain our finding in this post-reproductive age cohort.

Aim 3: Influence of resilience, optimism, and tolerance of uncertainty on health behavior intentions post disclosure of genetic variants of uncertain significance results.

Our findings showed there were no moderating effects of resilience, optimism, or tolerance of uncertainty on the predictor variables of perceived risk, perceived severity, perceived information value, or self-efficacy as hypothesized. Participants completed a baseline survey as part of enrollment to the parent (ClinSeq®) study that measured personality traits of resilience, dispositional optimism, and tolerance of uncertainty. Behavior intentions was moderately correlated with tolerance of uncertainty ($r=0.31$, $p = 0.147$), and weakly correlated with resilience ($r=0.11$, $p = 0.614$) and optimism ($r= 0.07$, $p = 0.745$). Interaction terms were generated between the personality traits and predictor variables to test whether the relation between a predictor variable and behavior intentions differed based on levels of each of the three personality traits. None of the interaction terms were statistically significant.

As a group, ClinSeq® participants have demonstrated enthusiasm for genome sequencing technology, displaying high levels of resilience, optimism, and tolerance of

uncertainty, and represent a segment of the public that are likely to pursue genetic technology for reasons beyond health concerns (Facio et al., 2011). The findings that these personality traits did not moderate behavior intentions were not anticipated. It is possible that these personality traits influence perceptions through an unknown mediating variable inherent to participants with the characteristics of our cohort, or that the small sample size was not adequate to detect their moderating effects.

Aim 4: Measurement of level of regret associated with decision to learn genetic variants of uncertain significance results 2 weeks post disclosure.

Sixty-eight participants completed the decision regret scale (DRS) used to measure regret associated with the decision to learn VUS results. The sample size of 68 is lower than reported for the other study aims. Measuring the regret associated with decision to learn VUS results was added to the study after data collection had begun.

With a mean of 12.40 (SD = 16.42), the scores were skewed towards no regret, with 51.5% of participants reporting no regret (score= 0), and 83.8% reporting no or mild regret (scores= 0-30). There was a significant correlation of decision regret to the sub-classes of VUS. Participants in the VUS-Low group reported higher regret scores on decision to learn their VUS results compared to those in the VUS-High group (r= -0.256, p= 0.035). The highest regret score of 62.5 was recorded in the VUS-Low group. Our findings show that participants distinguished between the two sub-groups of VUS based on the information they were given, as the difference in regret may be partly due to the subjective value attributed to each sub-group.

Discussion Summary

The purpose of this study was to describe the impact of disclosing genetic variants of uncertain significance results on health behavior intentions. To accomplish this goal, the interplay of uncertainty, perceptions, and behavior intentions was examined. The findings from this study extend current empirical knowledge in disclosure of uncertain genetic information to research participants. How sources and issues of uncertainty in genomics are conveyed, and how participants perceive them are relatively unknown, as there are no commonly accepted guidelines for providing clinical interpretation and recommendations for those receiving VUS results. This study extends the findings of hypothetical studies to actual participants facing these choices.

Using the HBM as a framework, the study findings describe the influence of perceived risk, perceived severity, perceived information value, and self-efficacy on health behavior intentions of research participants receiving cardiomyopathy-associated genetic VUS results. Perceived benefit, operationalized as value of information, was the strongest predictor of health behavior intentions, consistent with previously reported findings in this cohort of participants (Wright et al., 2014). Perceived risk, perceived severity, and self-efficacy were weak predictors of behavior intentions, with perceived severity not related in the predicted direction (negative correlation). The weak effects of perceived risk and severity on predicting health behavior intentions suggest that participants learning their cardiomyopathy-associated VUS results may not perceive themselves at risk for developing the condition, and may have determined that cardiomyopathy is not a serious condition that could affect their lives. Self-efficacy

levels were high in spite of its weak effect in predicting behavior intentions. The personality traits of resilience, optimism, and tolerance of uncertainty did not moderate the effects of the predictor variables on behavior intentions. The reported high levels of resilience, optimism, and tolerance of uncertainty, coupled with a yet to be identified potential mediating factor inherent to this cohort of respondents may have played a role in this unanticipated finding.

Participants in this study experienced little to no regret regarding decision to learn their VUS results. The sub-classification of VUS results into high and low groups significantly affected regret associated with recipients' decision to learn VUS results. This suggests that participants can receive and process genetic information with varying degrees of uncertainty. Following disclosure, participants in the VUS-Low group reported higher regret with decision to learn results compared to those in the VUS-High group. These results show that research participants deciding to learn VUS results may not regret their decision in spite of the uncertainty associated with the disclosed information. This minimal level of regret to disclosure of VUS results should positively inform future practice of returning sequencing information to research participants.

Strengths and Limitations

This study has several strengths. First, this was a non-hypothetical examination of the impact of disclosing genetic VUS on perceptions. Most of the studies investigating decisions and psychological implications related to learning VUS results in the literature are hypothetical in nature. Second, most of the research examining uncertain or

ambiguous genetic testing is conducted using *BRCA1/2* genes in hereditary breast and ovarian cancer patients and carriers. Comparison of the impact of VUS results disclosure across more genetic conditions, such as cardiomyopathy susceptibility, will help identify commonalities and differences in recipient experiences as future disclosure guidelines are developed. Understanding the expected benefits and value of returning VUS results to participants not only inform the on-going debate on the minimum results investigators have an obligation to return, it strengthens the social and moral contract between genetic researchers and participants (Facio et al., 2013). Disclosure of VUS results to actual participants will yield more valid associations and conclusions compared with previous studies of hypothetical VUS recipients. Third, using the genomics-first approach, this study utilized cardiomyopathy susceptible variants of uncertain significance from a large set of exome sequence data. The use of gene variant-to-phenotype (genomics-first) approach changes the focus from the usual diagnosis and treatment of disease (disease-first approach) to early identification and effective monitoring in otherwise healthy individuals. The availability and access to phenotypic data in the ClinSeq® cohort provides a unique opportunity to perform iterative clinical research to assess and monitor the pathogenicity of these VUS. Fourth, the response rate for survey completion was high (97.5%). Finally, although the intentions of participants to receive results associated with treatable or preventable conditions can be rationalized, the impact of uncertain genetic data on health behaviors is relatively unknown. This study describes perceptions of research participants and the value they attach to their uncertain genetic information.

There are also several limitations that are important to note. First, this was a correlational study; therefore, causal relationship cannot be drawn. Second, measures of perceived cardiomyopathy risk, perceived severity of cardiomyopathy, perceived value of VUS information, self-efficacy, and decision regret were collected using self-report measures in a cross-sectional manner, and therefore changes over time were not captured. Third, the small sample size may have limited the power to detect some relationships among the variables we examined.

Implications for Nursing Practice and Research

The implications of genetic testing for patients and research participants warrant further investigation as the speed of technological advancements outpace our ability to interpret generated information. Results of genetic testing returned to participants may provide some relief from fear of the unknown, and prompt individuals to make better health decisions (Tinkle & Cheek, 2002). Being at the forefront of care, nurses play a crucial role in identifying risk factors, gathering family histories, and providing genetic information and counseling to research participants, patients and their families. Nursing will need to develop better understanding of genetics and genomics to stay relevant in our role as patient advocates, and build on genetic knowledge that inform nursing practice and research. The challenges of uncertain genetic information disclosure are a call for nurses to understand the issues associated with informed consent in genetic testing and disclosure of VUS. As the focus of disclosure of genetic information include not only clinical but personal utility, nurses as patient advocates will have a role in

upholding the ethical principle of respect for persons by acknowledging the decisions of recipients of genetic VUS as autonomous agents. Genetics research is rapidly changing how we think about health as a nation. As testing becomes easily accessible, it is important that researchers understand perceptions and attitudes associated with genetic testing, especially as testing moves from mostly self-referred research populations to less self-selected samples (Coyne et al., 2003).

Ethical Implications

The mapping of the human genome has created new opportunities for genetic testing to potentially prevent disease. The ethical issues raised by this scientific trend primarily concern the management of personal genetic information with psychosocial implications. Genetic studies typically involve families, therefore research findings about individual subjects can have direct implications for other subjects without their approval. Some areas in genetics and genomics such as VUS interpretation and disclosure present issues for which no clear guidance can be given at this point because of limited risk-related evidence and lack of consensus on the appropriate course of action. Because of the uncertainties involved in genetic research, the rights and welfare of human subjects, such as psychological risk, personal utility of results, and respecting the wishes of the person, need to be part of an investigator's research plan. Scientific research has produced substantial social benefits and some troubling ethical questions. According to the Belmont Report, to show lack of respect for an autonomous agent is to

withhold information necessary for that agent to make a considered and valued judgment (Belmont Report, 1979).

Recommendations for Future Research

The results of this study provide several avenues for future research in disclosing genetic VUS results to research participants. First, our findings show that recipients of VUS results attach some benefit to receiving their results. In this sample of highly educated post-reproductive individuals, the perceived value of the information gleaned from VUS result disclosure was a strong predictor of behavior intentions. This finding cannot be immediately generalized. Further investigation of this work in cohorts with different characteristics is warranted. Second, the sub-levels of classifications within the VUS category showed significant difference in regret associated with decision to learn VUS results. Future attempts to clarify the uncertainty inherent to this class of genetic information should consider our sub-classification approach in their research design. Finally, the HBM dos not spell out the relationships among its variables, longitudinal designs that investigate the relation of perceived risk, severity, information value, and self-efficacy are necessary to better understand associations among these predictors of behavior. An experimental design with an intervention component specific to enhancing perceived benefits and self-efficacy should also be considered.

Conclusions

This dissertation describes the influences of some dimensions of the health belief model (HBM) on behavior intentions following disclosure of genetic VUS results. The uncertainty surrounding genetic information can affect disease perceptions and determine whether an individual act on the information. Our findings suggest that perceived benefits associated with receipt of uncertain genetic information is more likely to motivate recipients to act than discussions of possible risk and severity associated with their results. Additionally, research participants deciding to learn VUS results may not regret the decision despite the uncertainty associated with the disclosed information. This study contributes to an understanding of the experiences of individuals receiving uncertain genetic information.

References

Arar, N., Seo, J., Lee, S., Abboud, H.E., Copeland, L.A., Noel, P., et al. (2010). Preferences regarding genetic research results: comparing veterans and nonveterans responses. *Public Health Genomics*, 13, 431–439.

Bandura, A. (1977). Self-efficacy: toward a unifying theory of behavior change. *Psychological Review,* 84, 191-215.

Belmont Report. (1979). The Belmont Report: ethical principles and guidelines for the protection of human subjects of research. Retrieved May 8, 2014, from hhs.gov/ohrp/humansubjects/guidance/Belmont.html.

Biesecker, B.B., Klein, W., Lewis, K.L., Fisher, T.C., Frances, M., Biesecker, L.G., et al. (2014). How do research participants perceive "uncertainty" in genomic sequencing? *Genet Med*, 16(12), 977-980.

Bollinger, J.M., Scott, J., Dvoskin, R., Kaufman, D. (2012). Public preferences regarding the return of individual genetic research results: findings from a qualitative focus group study. *Genet Med*, 14, 451–457.

Brewer, N.T., Chapman, G.B., Gibbons, F.X., Gerrard, M., McCaul, K.D., & Weinstein, N.D. (2007). Meta-analysis of the relationship between risk perception and health behavior: the example of vaccination. *Health Psychol,* 26(2), 136-145.

Carpenter, C.J. (2010). A meta-analysis of the effectiveness of health belief model variables in predicting behavior. *Health Commun,* 25(8), 661-669.

Coyne, J.C., Kruus, L., Racioppo, M., Calzone, K.A., & Armstrong, K. (2003). What do ratings of cancer-specific distress mean among women at high risk of breast and ovarian cancer? *Am J Med Genet,* 116A, 222-228.

Facio, F.M., Eidem, H., Fisher, T., et al. (2013). Intentions to receive individual results from whole-genome sequencing among participants in the ClinSeq study. *Eur J Hum Genet,* 21, 261–265.

Grembowski, D., Patrick, D, Diehr, P., Durham, M., Beresford, S., Kay, E., et al. (1993). *J Health Soc Behav,* 34(2), 89-104.

Harris, E.D., Ziniel, S.I., Amatruda, J.G., et al. (2012). The beliefs, motivations, and expectations of parents who have enrolled their children in a genetic biorepository. *Genet Med,* 14, 330–337.

Harrison, J. A., Mullen, P. D., & Green, L. W. (1992). A meta-analysis of studies of the health belief model with adults. *Health Education Research*, 7, 107–116.

Hawkins, R.M. (1992). Self-efficacy: a predictor but not a cause of behavior. *J Behav Ther Exp Psychiatry*, 23(4), 251-256.

Lindor, N.M., Goldgar, D.E., Tavtigian, S.V., Plon, S.E., & Couch, F.J. (2013). BRCA1/2 sequence variants of uncertain significance: a primer for providers to assist in discussions and in medical management. *The Oncologist*, 18, 518-524.

Lindor, N.M., Guidugli, L., Wang, X., Vallée, M.P., Monteiro, A.N.A., Tavtigian, S., et al. (2012). A review of a multifactorial probability-based model for classification of BRCA1 and BRCA2 variants of uncertain significance (VUS). *Hum Mutat*, 33, 8-21.

Quarta, G., Papadakis, M., Donna, P.D., Maurizi, N., Iacovoni, A., Gavazzi, A., et al. (2016). Grey zones in cardiomyopathies: defining boundaries between genetic and iatrogenic disease. *Nat Rev Cardiol,* doi: 10.1038/nrcardio.2016.175. [Epub ahead of print]

Rosenstock, I. M., Strecher, V. J., & Becker, M. H. (1988). Social learning theory and the health belief model. *Health Education Quarterly*, *15*, 175–183.

Sen-Chowdhry, S., Jacoby, D., Moon, J.C., & McKenna, W.J. (2016). Update on hypertrophic cardiomyopathy and a guide to the guidelines. *Nat Rev Cardiol*, 13(11), 651-675.

Strecher, V.J., DeVellis, B.M., Becker, M.H., & Rosenstock, I.M. (1986). The role of self-efficacy in achieving health behavior change. *Health Educ Q,* 13(1), 73-92.

Tinkle, M.B., & Cheek, D.J. (2002). Human genomics: challenges and opportunities. *J Obstetrics, Gynecologic, and Neonatal Nursing,* 31, 178-187.

Wade, C.H., Shiloh, S., Woolford, S.W., Roberts, J.S., Alford, S.H., Marteau, T.M., et al. (2011). Modeling decisions to undergo genetic testing for susceptibility to common health conditions: an ancillary study of the Multiplex Initiative. *Psychol Health,* 27, 430-444.

Weinstein, N.D., Kwitel, A., McCaul, K.D., Magnan, R.E., Gerrard, M., & Gibbons, F.X. (2007). Risk perceptions: assessment and relationship to influenza vaccination. *Health Psychol,* 26(2), 146-151.

Wright, M.F., Lewis, K.L., Fisher, T.C., Hooker, G.W., Emanuel, T.E., Biesecker, L.G., et al. (2014). Preferences for result delivery from exome sequencing/genome sequencing. *Genet Med,* 16, 442-447.

APPENDIX

Appendix A: Recruitment Letter

Dear _____,

I am writing to invite you to participate in a study about genetic variants of uncertain significance results. A student investigator from the Johns Hopkins School of Nursing working with the ClinSeq® team at the National Human Genome Research Institute is conducting the study.

The main goal of this study is to examine the impact of receiving a certain classification of genetic test results known as Variants of Uncertain Significance (VUS) on health behaviors. You are being contacted because a gene variant of uncertain significance (VUS) has been identified within your DNA sequence analysis.

There is a shortage of research in genetics that examine the impact of uncertain genetic information disclosure and how an individual may choose to use the information. In the future, we hope that the findings of this study will help researchers and clinicians effectively disclose and manage this kind of result. This study may generate useful information that helps us understand the impact of these VUS results in other genetic conditions as well.

If you are willing to take part in this study or are interested in receiving more information about this study, please contact Tokunbor Lawal @ XXX-XXX-XXXX. We will follow up our invitation to participate with a phone call to address any questions within a few days. Thank you for your consideration and we look forward to hearing from you.

Sincerely,

Tokunbor Lawal, RN, MSN

Les Biesecker, MD

Appendix B: Initial Contact Script

[TAL]: *Hi, Thanks for calling! I'm Tokunbor (Toks) Lawal and I am a student at the Johns Hopkins University School of Nursing and the National Institutes of Health in Maryland. As you probably read, we are conducting a study on the impact of receiving a genetic VUS result. I'm interested in this topic because I think it's important to understand how this sort of test results affect people. Understanding this experience can ultimately help health providers be more effective in disclosing these results in the future. Taking part in this study is completely voluntary and you can stop at any time. Are you still interested in learning more about this study?*

If the answer is no: *Thank you for your time! (Probe for details on why participant does not want result) Have a nice day.*

If the answer is yes: *Now, I would like to ask you a few questions to make sure you are eligible to participate and tell you a bit more about the study.*

[TAL]: *Are you able to consent for yourself to participate in this study?*

[TAL]: *Are you over 45 years old?*

[TAL]: *Are you currently enrolled in the ClinSeq study at the NIH?*

Verbal Informed Consent

We are currently returning people's results to them as part of a study to examine the impact of returning results with ambiguous meaning to participants. These results are also known as variants of uncertain significance or VUS for short. You are being invited to participate in this study because you are enrolled in the ClinSeq project, and have a genetic VUS result that can be returned to you soon. I will describe the study so that you can decide whether you are willing to participate. Taking part in this study, like any study, is voluntary. You may choose not to take part or withdraw from the study at any time.

If you choose to participate, you will be asked to complete an online survey to assess how you view risk, and your feelings about genetic information. You will also be asked to come in to the NIH for a visit lasting 1-2 hours. When you arrive, you will meet with the investigator to learn and discuss your result. You will receive a copy of your testing result and a written letter summarizing your result at the end of your visit. You will be asked to complete a survey 2 weeks after you get your result. We will be calling or sending you reminders to complete this survey.

The risks and discomforts of this study are most likely to be psychological. For example, some people may become anxious or upset to learn about gene variants that have ambiguous or uncertain meanings.

Although potential benefits to your health could develop from participating in this research study, you do have an actual opportunity to help us learn more about the impact of returning ambiguous testing results from genetic sequencing to research participants.

It is your choice whether or not to be in this study. You may stop participating in this study at any time, while remaining in the ClinSeq study. If you choose to withdraw, you may request that we destroy the information we may have collected about you.

Only members of the ClinSeq research team will know that you are participating in this study. Data from this study will be identified with a code number and not your name. The key for this code will be stored in a secure database. Data that we collect from you may be shared with investigators we collaborate with in the future. This data would be linked with other information, such as your age, gender and ethnicity, but not your name, address or phone number.

You will not receive payment for taking part in this study.

Would you be willing to participate in this project?

☐ Yes: Great, thank you for your interest. Your visit is expected to last between 1 and 2 hours, and will take place at the NIH. *Proceed with visit scheduling.* A few days prior to your visit, you will receive a packet that will contain information on your appointment time and location. In the meantime, please feel free to call us with any questions or concerns. We are looking forward to seeing you soon. (Confirm or obtain updated contact information if not available on file)

☐ No: That's okay. *Probe for details on why participant does not want to participate to ensure that they have not misunderstood the project. Record their responses on this form.*

[TAL]: *Thank you very much and I look forward to meeting you on the* _____.

Appendix C: Standard Protocol for VUS Disclosure

Standard Script

Script: *Mr (Ms.)_____ Thank you for making time to be here to receive this result in person.*

- Introduction & Contracting
    - Review ClinSeq goals
    - Discuss typical criteria for result return
    - Specify differences for the results received today
    - Ensure that participant wants to receive this type of result (re-consent)
    - Outline flow of session
- Review of genetics
    - Basic principles of how mutation causes disease
    - Autosomal dominant inheritance pattern
    - Penetrance
- Review of result report
    - Explanation of report layout
    - Description of pathogenicity rating (**see visual below**)
- Overview of cardiomyopathy
    - Definition (see visual below)
    - Age of onset and symptoms
    - Diagnosis
    - Treatments
- Recommendations
    - Sharing with physicians, consider seeking out cardiologist
    - Sharing with family members
- Counseling (by genetic counselor if requested or need is recognized)
    - Initial reactions to results
    - Help deciding what to do with information
    - Processing emotional reactions to results
    - Discussion of risk perceptions

Appendix D: Samples of Visual Aids

How to Read Your Result

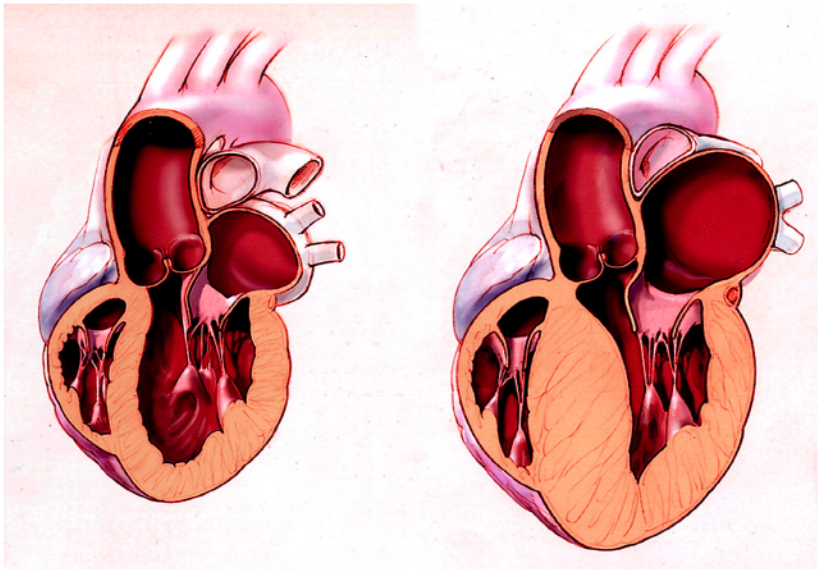
Predicted pathogenicity is the likelihood that the genetic change you have would cause the condition. This rating is based on the quantity and quality of information we have about the genetic change. The table below explains the possible ratings:

Predicted pathogenicity	Variant of Uncertain Significance	Likely Pathogenic	Pathogenic
Means that the variant you have.....	May or may not cause a health condition	Likely causes a health condition	Is known or highly likely to cause a health condition
Means that the information we have about this variant is.....	Limited → Extensive		

Low — High

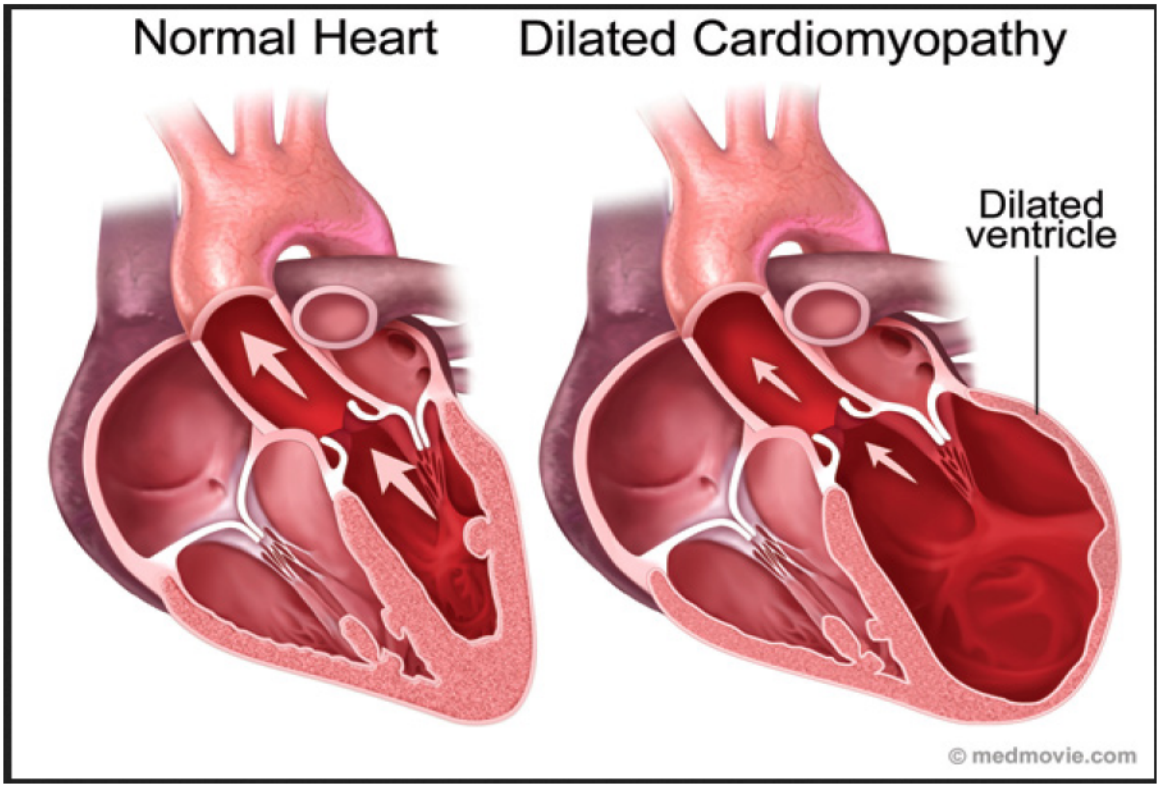
Reported in this study — Reported in ClinSeq

Schematic diagram of a patient with a normal heart (left) and a patient with hypertrophic cardiomyopathy (right).



Nishimura R A et al. *Circulation.* 2003;108:e133-e135

American Heart Association.


Normal Heart
Dilated Cardiomyopathy
Dilated ventricle
© medmovie.com

APPENDIX E: STUDY INSTRUMENTS

*** 1. Please enter your Custom ID**

Custom ID

2. Please enter your initials:

3. Please enter today's date below.

	MM	DD	YYYY
Today's Date:	/	/	

4. We need to first assess your understanding. What do you understand your VUS result to mean?

INTENTIONS

* 5. By participating in the ClinSeq study and having your genome sequenced, you have learned about a gene variant that may predispose you to cardiomyopathy

	Definitely no	Probably no	Unsure	Probably yes	Definitely yes
I intend to seek more information about this result in the future	○	○	○	○	○
I intend to share this result with my healthcare provider	○	○	○	○	○
I intend to use this result to change my lifestyle/health behaviors (diet, exercise, screening)	○	○	○	○	○
I intend to share this result with my family members	○	○	○	○	○

6. If your parents are still living, do you intend to share this result with them?

- ○ Yes
- ○ No
- ○ My parents are not still living

7. If you have a spouse/partner, do you intend to share this result with them?

- ○ Yes
- ○ No
- ○ I do not have a spouse/partner

8. If it applies to you, please indicate which family members you intend to share this result with

	How many do you have?	How many do you intend to share this result with?
Brother Sister		
Daughter		
Son		

Please list any other relatives who you intend to share this result with:

*** 9. Please indicate how likely it is that you will:**

	Extremely unlikely	Very unlikely	Somewhat unlikely	Unsure	Somewhat likely	Very likely	Extremely likely
Seek more information about this result in the future?							
Share this result with your healthcare provider?							
Use this result to change your lifestyle/health behaviors?							
Share this result with your family members?							

10. If you shared your result, how did you describe it to your family members?

PERCEIVED RISK

* 11. How likely are you to get cardiomyopathy in your lifetime?

- Extremely unlikely
- Very unlikely
- Somewhat unlikely
- Neither unlikely nor likely
- Somewhat likely
- Very likely
- Extremely likely

*** 12. How certain are you about your answer?**

Not at all certain		Somewhat certain		Very certain		Extremely certain
1	2	3	4	5	6	7

*** 13. How confident are you that the estimate you gave in Q11 above is accurate? (that it reflects your actual risk)**

Not at all confident		Somewhat confident		Very confident		Extremely confident
1	2	3	4	5	6	7

14. Compared to other people your age and sex, how likely are you to get cardiomyopathy in your lifetime?

Much less likely than the average person			About as likely as the average person			Much more likely than the average person
1	2	3	4	5	6	7

15. How certain are you about your answer?

Not at all certain		Somewhat certain		Very certain		Extremely certain
1	2	3	4	5	6	7
○	○	○	○	○	○	○

16. How confident are you that the estimate you gave in Q14 above is accurate? (that it reflects your actual risk)

Not at all confident		Somewhat confident		Very confident		Extremely confident
1	2	3	4	5	6	7
○	○	○	○	○	○	○

17. In Q11 and Q14, you indicated your beliefs about how likely it is that you will get cardiomyopathy. However, at a gut-level, you might feel more or less vulnerable than your response above suggests. How likely do you feel it is that you will get cardiomyopathy in your lifetime?

- ○ Extremely unlikely
- ○ Very unlikely
- ○ Somewhat unlikely
- ○ Neither likely nor unlikely
- ○ Somewhat likely
- ○ Very likely
- ○ Extremely likely

18. How certain are you about your answer?

Not at all certain		Somewhat certain		Very certain		Extremely certain
1	2	3	4	5	6	7
○	○	○	○	○	○	○

19. How confident are you that the estimate you gave in Q17 above is accurate? (that it reflects your actual risk)

Not at all confident		Somewhat confident		Very confident		Extremely confident
1	2	3	4	5	6	7
○	○	○	○	○	○	○

PERCEIVED VALUE OF INFORMATION

*** 20. Through ClinSeq you learned you have a variant related to cardiomyopathy. Please respond to the following statements about that result.**

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
My results will help reduce my chances of getting heart disease	○	○	○	○	○
My results will help reduce my chances of developing cardiomyopathy	○	○	○	○	○

*** 21. Please indicate how strongly you agree with each statement about your result.**

	Strongly disagree				Strongly agree
My sequence result is valuable for maintaining my future health	○	○	○	○	○
My sequence result is valuable for maintaining my family's future health	○	○	○	○	○
My sequence result is useful to my physician	○	○	○	○	○
I trust my sequence result	○	○	○	○	○
My sequence result gives clear answers about my future health	○	○	○	○	○
My sequence result gives clear answers about my family's future health	○	○	○	○	○

*** 22. Which of the following is the best any person could hope for as a result of getting this type of sequence result?**

- ○ Cardiomyopathy will be cured and future problems will be prevented
- ○ Cardiomyopathy complications will be reduced and future problems will be prevented
- ○ Cardiomyopathy complications will not get any worse and future problems will be prevented
- ○ Cardiomyopathy complications will not improve but future problems will be prevented
- ○ Cardiomyopathy complications will not improve but some future problems will be prevented
- ○ Cardiomyopathy complications will not improve and future problems will not be prevented
- ○ Don't Know
- ○ Unsure

PERCEIVED SEVERITY

*** 23. Please rate how strongly you agree with each of the following statements.**

	Strongly disagree	Disagree	Unsure	Agree	Strongly agree	N/A
My feelings about myself would change if I develop cardiomyopathy	○	○	○	○	○	○
If I had cardiomyopathy my career would be endangered	○	○	○	○	○	○
Cardiomyopathy would endanger my marriage or other significant relationships	○	○	○	○	○	○
Cardiomyopathy is a hopeless disease	○	○	○	○	○	○
My financial security would be endangered if I develop cardiomyopathy	○	○	○	○	○	○
Problems I would experience from cardiomyopathy would last a long time	○	○	○	○	○	○
If I develop cardiomyopathy, it would be more serious than other diseases	○	○	○	○	○	○
My whole life would change if I develop cardiomyopathy	○	○	○	○	○	○

PERCEIVED SELF-EFFICACY

*** 24. Please rate your response to the following statements**

	Strongly agree	Agree	Unsure	Disagree	Strongly disagree
I feel confident and competent in my ability to pursue health behaviors related to my variant for cardiomyopathy	○	○	○	○	○
I am confident in my ability to use medical resources to manage my variant for cardiomyopathy	○	○	○	○	○

Decision to Receive VUS Result

25. Please think about the decision you made about receiving this genetic VUS result. Please show how strongly you agree or disagree with these statements about your decision.

	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
It was the right decision	○	○	○	○	○
I regret the choice that was made	○	○	○	○	○
I would go for the same choice if I had to do it over again	○	○	○	○	○
The choice did me a lot of harm	○	○	○	○	○
The decision was a wise one	○	○	○	○	○

Satisfaction

26. In what ways were you satisfied with the result you received? In what ways were you not?

APPENDIX F: Summary letter (Sample) sent 2 weeks post disclosure

Dear Mr. XXXXXX,

You came to the National Institutes of Health (NIH) on XX/XX/XX to receive a result from your exome sequencing (ES) genetic testing. You had ES through a research project at the National Institutes of Health (NIH) called the ClinSeq® study. The result was delivered to you in the context of a side research project we are conducting. This side project aims to examine the impact of your genetic testing result on your health behavior.

Your Result & Its Meaning

As detailed in your Secondary Alteration Report (which you received a copy of at your visit), you have a genetic change in a gene called *TTN*. Some people with changes in this gene have been reported with health problems, including dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), centronuclear myopathy (CNM), tibial muscular dystrophy, neuromuscular disorder, and sudden cardiac death. Other health problems have also been reported in people with changes in this gene, but we will not describe them in detail here because they have only been reported in a few people and there is insufficient scientific evidence about whether changes in this gene are associated with an increased risk for these conditions or because they have been only associated with one specific change which is not the one you have.

Our report classifies the genetic change you have as a variant of uncertain significance (VUS). This means that **we don't know at this time whether the change increases your risk to develop DCM, ARVC, CNM, tibial muscular dystrophy, neuromuscular disorder and/or sudden cardiac death or not**. We do not have enough evidence at this time to say whether this genetic change has implications for your health, or puts you or your family members at risk for these conditions. This result does not diagnose you with DCM, ARVC, CNM, tibial muscular dystrophy, neuromuscular disorder or sudden cardiac death.

For the purposes of this research study, we also evaluated your genetic change using an algorithm designed to rate the impact of a genetic change on a gene's function. Based on the results of this algorithm, your result is considered a VUS-Low. This means that the algorithm predicts the effect or impact of your genetic change on how your gene works to be low when compared with other changes classified as a VUS. The designation of VUS-Low is based on one algorithm's prediction and may not be accurate. Therefore, this designation is for research purposes only and should not be used to direct your care.

Dilated Cardiomyopathy (DCM) and Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)

Cardiomyopathy is a disease of the heart muscle that makes it difficult for the heart to perform its main function of pumping blood to the rest of the body. This can lead to symptoms such as: feeling tired (especially when exercising), fainting, or having irregular heartbeats. Over time, the damage to the heart becomes more severe and can lead to heart failure or sudden death. Sometimes the first symptom a person experiences is sudden death. Some people with cardiomyopathy have no signs or symptoms in the early stages of the disease. For others, the disease develops quickly; symptoms are severe, and serious complications may occur. Two types of cardiomyopathy have been associated with changes in the *TTN* gene: DCM and ARVC.

DCM is a condition in which the heart muscle is thinner and weakened. As a result, the heart cannot pump enough blood to the rest of the body. This condition may occur at any age, but is most often diagnosed in the 40's – 60's.

ARVC is a rare form of cardiomyopathy in which the heart muscle is replaced by fat and/or scar tissue, leading to dilation and poor contraction. ARVC often leads to abnormal heart rhythms that increase the risk of sudden cardiac death. Although a family history of ARVC is present in almost 50% of cases, it can also occur in individuals with no family history.

In order to determine whether someone has DCM, or ARVC they must have testing to look at their heart's structure and function, which could include an echocardiogram, cardiac MRI, EKG, or exercise stress test. The results of these tests must be evaluated in the context of the person's medical and family histories.

Centronuclear Myopathy (CNM)

This rare condition is characterized by muscle weakness and wasting in the muscles used for movement (skeletal muscles). Individuals with CNM and alterations in the *TTN* gene begin experiencing muscle weakness in childhood. Muscle weakness worsens over time and can lead to delayed development of motor skills in childhood, muscle pain during exercise, and difficulty walking. Breathing and feeding difficulties have been reported in severely affected individuals.

Most people with CNM associated with changes in the *TTN* gene have changes in **both** copies of the gene. However, one individual with a change in only one copy of the gene did have mild symptoms of the condition.

People with changes in the *TTN* gene have also had other types of muscle disease. Those individuals have muscle weakness and wasting, but the severity of their symptoms and age of onset can be variable.

Tibial Muscular Dystrophy

This condition affects the muscle at the front of the lower leg, with the first sign of muscle weakness and wasting appearing after age 35. This muscle controls the up-and-down movement of the foot; therefore, weakness of this muscle may lead to difficulty with walking. The muscle weakness worsens very slowly and may affect muscles that help extend the toes. Although this condition is most common in people of Finnish descent, it has also been identified in several European families without Finnish ancestry.

Neuromuscular Disorder

One paper evaluated 23 individuals with various types of neuromuscular disorders, which are conditions that affect the way that the muscles and nerves work and which cause a broad range of symptoms. Although a small number of these individuals had one or more changes in the *TTN* gene, many of them also had changes in other genes that could be related to their health conditions. Therefore, the association of variants in *TTN* and neuromuscular disorder is still questionable and requires more research.

Sudden cardiac death

Sudden cardiac death is an unexpected death in a person due to heart disease. This is different from a heart attack caused by blockage in one or more of the coronary arteries. Sudden cardiac death occurs due to problems with the electrical system of the heart, which cause the heart beat to become irregular. Sudden cardiac death may occur following symptoms of chest pain, heart palpitations, fainting or near-fainting, or without any preceding symptoms. It occurs most frequently in adults in their 30's and 40's, and affects men more often than it does women.

One paper evaluated 29 children with sudden cardiac death. Although a small number of these individuals had one or more changes in the *TTN* gene, many of them also had changes in other genes that could be related to their health conditions. Therefore, the association of variants in *TTN* and sudden cardiac death (in the absence of DCM or ARVC) is still questionable and requires more research.

Inheritance of the *TTN* Gene

As you may recall, you have two copies of most genes in your body. One copy is inherited from your mother, and the other is from your father. In some cases, having a change in one copy of the gene may cause the gene to stop working properly and may put you at an increased risk for developing a health condition. This is called a dominant inheritance pattern. In other cases, you need to have changes in both copies of the gene in order to be at increased risk for developing a health condition, which is called a recessive inheritance pattern.

The result you received at your visit is for a gene that predisposes to some conditions that are passed on in a dominant pattern and other conditions that are passed on in a recessive pattern. Our testing found that you have a change in one copy of the *TTN* gene. Current evidence indicates that this **may or may not** impact your risk to develop any of the listed

conditions. Since you have one changed copy of the *TTN* gene, each of your siblings and children has a 50% chance to have also inherited a changed copy of the gene.

Recommendations

At this time, there are no established guidelines about whether people with a VUS in the *TTN* gene (like you) should be screened for cardiomyopathy, muscular dystrophy or myopathy. If you are concerned about your risk to develop these conditions, we suggest that you share this information with your doctors. They can determine how best to incorporate this finding into your health care, along with your personal and family history.

Testing Limitations

There are several limitations of this testing that you should know about. First, this testing is not designed to find all possible genetic changes that put you and your family members at risk for health problems. For this result, 41 genes that are associated with cardiomyopathy were reviewed. Current sequencing technology cannot detect all of the possible changes that can occur in these 41 genes. Also, changes in these genes may not be interpretable based on current knowledge. Second, there are many other genes that we did not examine in this study. Therefore, the absence of a genetic testing result in the unexamined genes does not exclude the possibility of having an affected family member in the future.

Survey Completion Reminder

We very much appreciate your ongoing participation in ClinSeq® and in this side project on the impact of returning genetic variants of uncertain significance. We ask that you please complete a survey for us on your experiences receiving this result.
You can find the survey at:

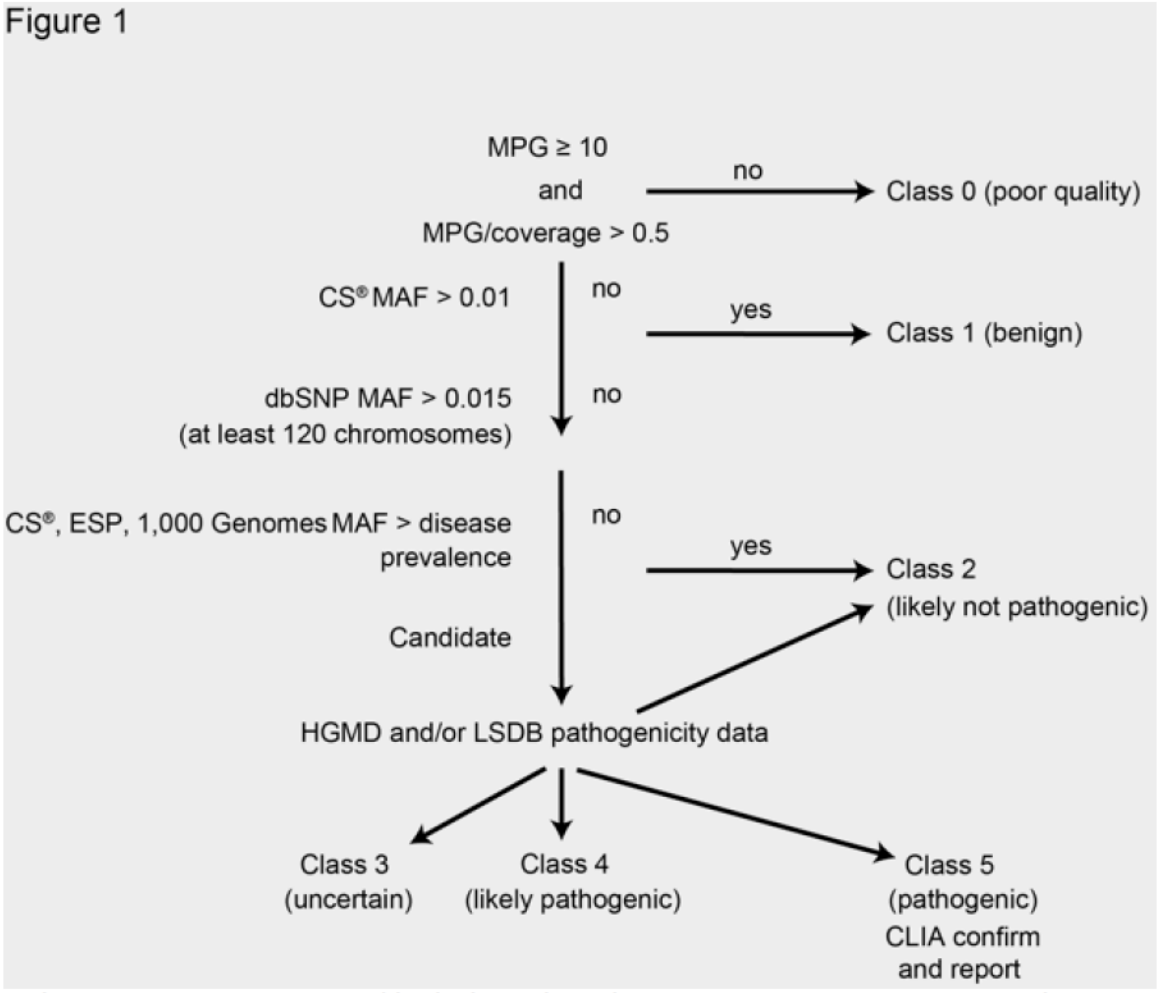
https://www.surveymonkey.com/r/VUS2WKPOST
and your personal access code is: [XXXX].

We will also be contacting you in 8 months to complete one more survey about your experience receiving these results.

Please remember that we will continue to share more results with you in the coming months and years. We will contact you with those results as they become available. Please call us if you or your doctors have questions or if you need any referrals. Thank you again for your time and participation in the study.

Sincerely,

Appendix G: Framework for variant interpretation for cardiomyopathy variants from Ng et al., 2013



MPG = most probable genotype score; CS = ClinSeq; dbSNP = Single Nucleotide Polymorphism Database; ESP = Exome Sequencing Project; MAF = minor allele frequency; HGMD = Human Gene Mutation Database; LSDB = locus-specific database.

CURRICULUM VITAE

PERSONAL DATA

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104 Harbinger Drive
Derwood, MD 20855
E-mail: tlawal1@jhu.edu

EDUCATION AND TRAINING

Institution	Degree	Year
Johns Hopkins University School of Nursing Baltimore, MD	Doctor of Philosophy in Nursing	2011-Present
Marymount University School of Health Professions Arlington, VA	Master of Science in Nursing Family Nurse Practitioner	2011
Georgetown University School of Nursing & Health Studies Washington, DC	Bachelor of Science in Nursing	2007
University of Maryland College of Life Sciences College Park, MD	Bachelor of Science in Biology	1999

CURRENT LICENSE AND CERTIFICATION

Year	Licensing Body Expiration	Number
2007-present	Maryland Board of Nursing 3/28/2018	R177887

PROFESSIONAL EXPERIENCE

Position	Institution	Years
Pre-doctoral Fellow	National Institute of Nursing Research National Institutes of Health Bethesda, MD	2011-present
Clinical Research Nurse	Clinical Center National Institutes of Health Bethesda, MD	2007-2011
Research Technician	Covance Laboratories, Inc. Vienna, VA	2001-2007

APPOINTMENTS

Position	Institution	Years
Lieutenant Commander	United States Public Health Service	2008-present

SCHOLARSHIP

Publications

Gaston-Johansson, F., Haisfield-Wolfe, M.E., Reddick, B., Goldstein, N., & **Lawal, T.A**. (2013). Understanding the Relationship among Coping Strategies, Religious Coping, and Spirituality in African American Women with Breast Cancer Receiving Chemotherapy. *Oncology Nursing Forum,* 40(2), 120-31.

Presentations

Lawal, T.A. & Biesecker, L.G. (Poster Presentation) Decision Regret Associated with Disclosing Genetic Variants of Uncertain Significance Results. 90th meeting of the National Advisory Council for Nursing Research, Bethesda, MD, September 13, 2016.

Lawal, T.A. (Oral Presentation) The Impact of Genetic Variants of Uncertain Significance Disclosure on Health-Related Behaviors. The 85th meeting of NINR National Advisory Council for Nursing Research, Bethesda, MD, September 16, 2014.

EDUCATIONAL ACTIVITIES

Semester	Number & Name of Course	Role
Spring 2012	NR110.491 - Dying & Death, Graduate, 11 students	Teaching Assistant
Spring 2012	NR110.500 – Philosophical, Theoretical, and Ethical Basis of Advanced Practice Nursing, Graduate, 21 students	Teaching Assistant

ACADEMIC SERVICE

Years	Committee	Role
2012-2013	Doctoral Student Organization, Johns Hopkins University School of Nursing	President
2012-2013	William H. Welch Medical Library Advisory Committee	Student Representative